VOLUME 94 • NOVEMBER 1957 • NUMBER 5

A.M.A. JOURNAL OF

DISEASES OF CHILDREN

Editorial

Presidential Address, Society for Pediatric Research
William M. Wallace

Society for Pediatric Research

Proteus Meningitis
Hyman C. Tolmas and Jacqueline Winter

Abstracts from Current Literature

News and Comment

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A. M. A.
Journal of Diseases of Children

VOLUME 94 NOVEMBER 1957 NUMBER 5

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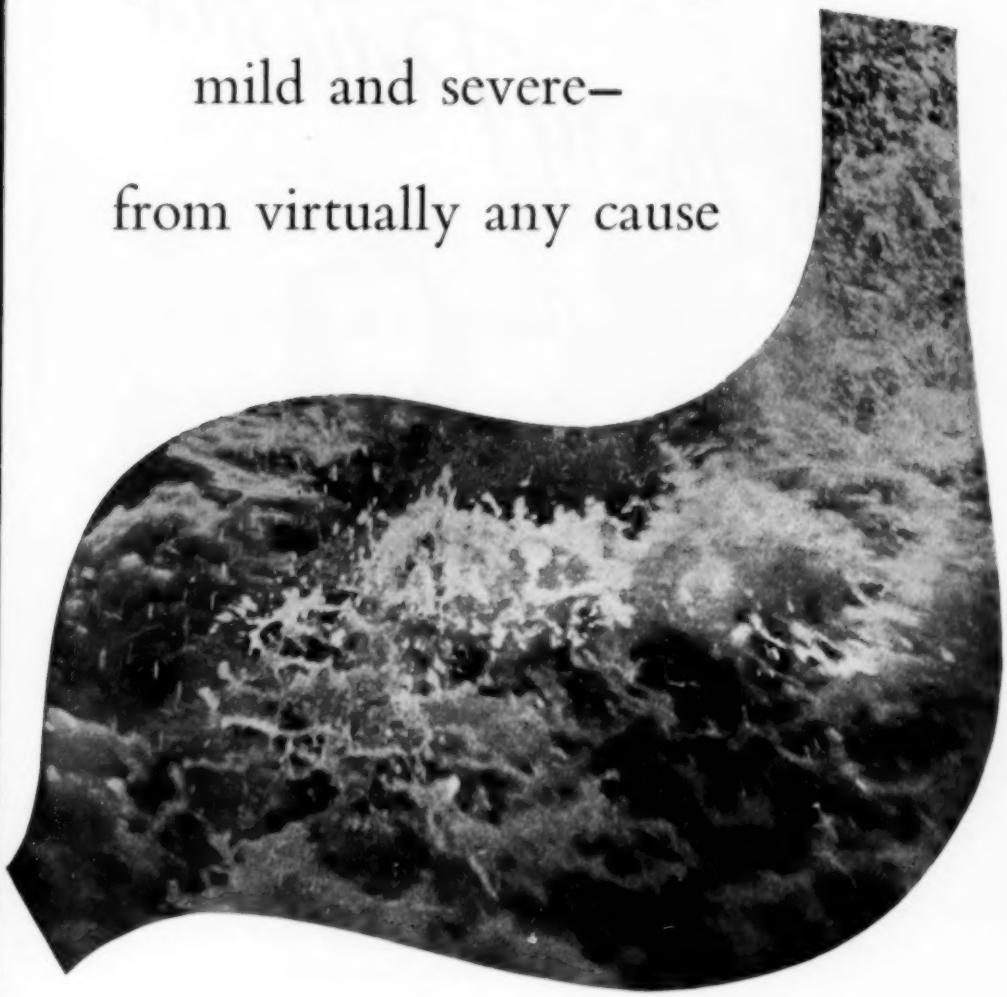

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(1) Smith, N. J., and Rosello, S.: J. Clin. Nutrition *1*:275, 1953.

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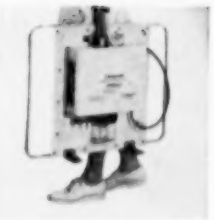

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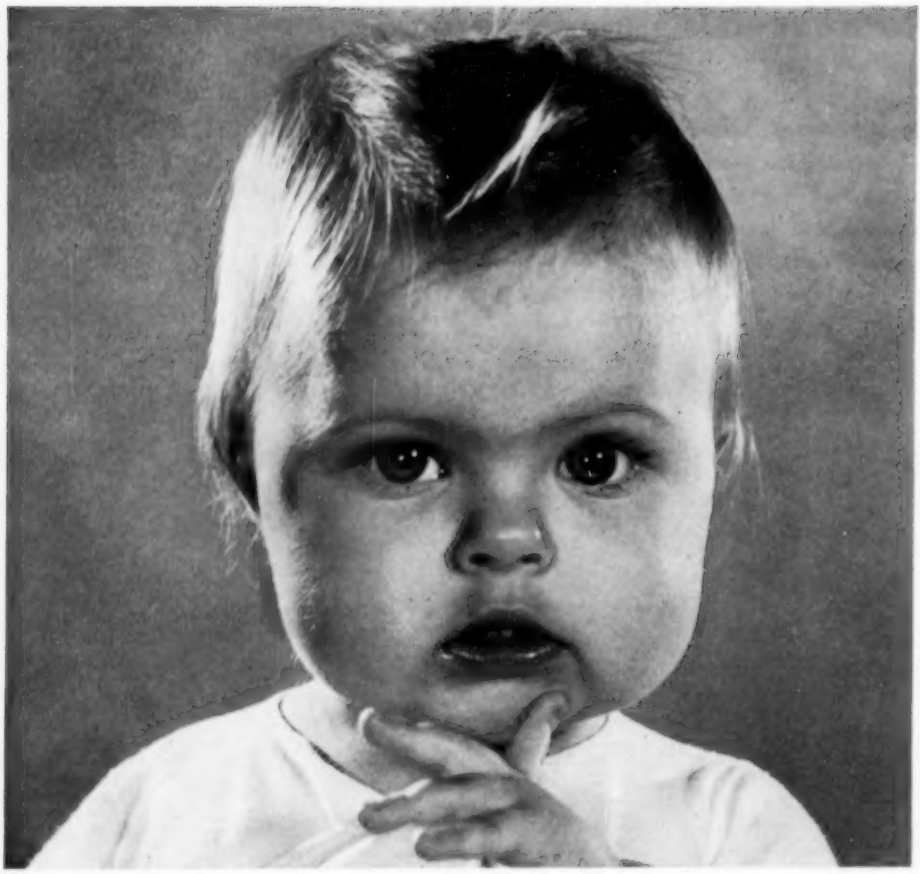

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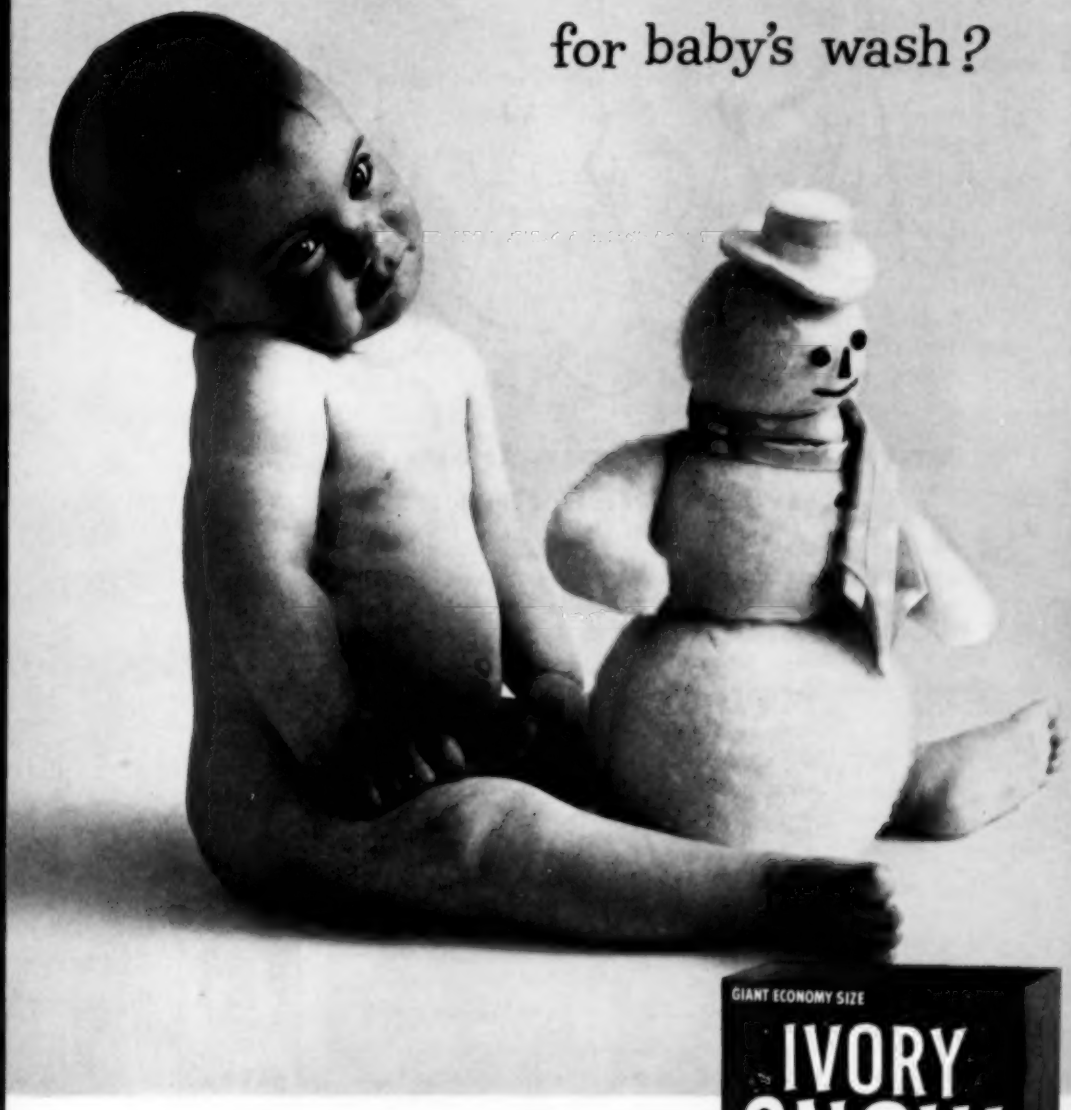

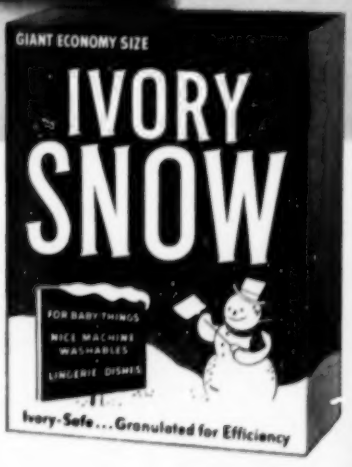

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Editorial

With this issue Dr. Richard L. Day assumes the position of Chief Editor of the A. M. A. JOURNAL OF DISEASES OF CHILDREN. The editors of the JOURNAL feel very fortunate in having Dr. Day accept this position because of his extensive background in clinical pediatrics, education, and research.

Dr. Day was born in New York City, graduated from Harvard Medical School, and received his hospital training at the Hartford Hospital in Hartford, Conn. and at the Willard Parker Hospital and The Babies Hospital in New York City. After serving in the Department of Pediatrics of Cornell University and then Columbia, Dr. Day was appointed Professor of Pediatrics and Chairman of the Department in the newly reorganized Medical School of the State University of New York at New York in 1953, which position he still holds. He is a member of the American Medical Association, the American Pediatric Society, The Society for Pediatric Research, The American Society for Human Genetics, and The Society of Experimental Biology and Medicine. He joined the editorial staff of the A. M. A. JOURNAL OF DISEASES OF CHILDREN in 1954.

It was with regret that the past Chief Editor felt it necessary to relinquish this position owing to the pressure of other work. However, this regret was tempered by the realization that the direction of the future of the JOURNAL would be in such capable hands.

Richard L. Day, M.D.

Presidential Address, Society for Pediatric Research

WILLIAM M. WALLACE, M.D., Cleveland

The first item on this program is an extravagant exaggeration. I have neither the qualifications nor the desire to give an address. The greater part of my time in this Society has been spent in the laboratory tilling an astonishingly small piece of ground. From this narrow position I find myself in no position to survey the field of pediatric research in such a way as to illuminate its past nor to point out its future course. Neither would I dare assay, as many have done, to reanalyze my own experimental affairs in light of that doubtful thing called maturity. There is too much left unanswered, and too many nimble minds have beaten me to my cherished aims. During the later years of membership in this Society, I fell into the trap of "running a department." This is too wintry a subject to discuss in California in the month of June. Presidential addresses have long been a custom in societies such as this and, like all customs, require the skills of an anthropologist to unravel their origins. Earlier this spring I obliquely questioned my mentor and friend, Dr. James L. Gamble, concerning a suitable topic for such an occasion. When I received the challenging and disconcerting answer that I should "polish a few platitudes," I pursued the conversation no further. I have listened to many such addresses during my period of membership in this and other societies of investigators. It is on their credit side that none have taken the easy path of self-congratulation. They have questioned and challenged the larger issues—our slowness to understand, our mistakes, and the frailty of our intellectual techniques. Of late years it has been usual to protest our alleged lack of fiscal support by the community. Perhaps these protests have led to the logarithmic increase in the past ten years of the number of papers submitted to this Society for presentation. One must always ask just how much lushness we deserve. I would insert parenthetically at this point that we must soon examine the method for conducting our program lest the mass of abstracts reach the critical size that ends in self-destruction.

I have never been quite at ease during public discussion of the broad and large issues that so often seem required of presidential orations. Therefore, I will shun consideration of such items and try to speak informally of the nature of the nonscientific support that this Society has generously given for so long to me and all of its membership. Were one only to attend the formal sessions of this Society, there would be no doubt left that research proceeds in a logical and precise fashion, that all such activity pays off in a productive fashion, and that presenters of papers rejoice in the security of unsurpassable ego strength. That many data remain as inscrutable numbers in a notebook, that cherished hypotheses turn out to be incorrect, and that excessive vasomotor activity invariably accompanies the polished presentation is learned and shared during journeys to and from the meetings, in pre- and postsession walks in the artificially exquisite surroundings that few of us are in the economic position to enjoy, in bars off the boardwalk, or in hotel bedrooms. My earliest pilgrimages to these meetings were usually made in unfashionable vintage automobiles that invariably detached their piston rods while climbing the Poconos or while going too

Presented at Carmel, Calif., June 19, 1957.

Received for publication July 8, 1957; accepted July 15.

fast on the New Jersey Turnpike. This was always annoying and led to complicated schemes as to how to pay for the repairs. Paradoxically, these catastrophically extended journeys paid the dividend of closer friendship with colleagues in this Society as well as the opportunity to air and detoxify many of the anxieties that faced us all as investigators. Thanks to the generosity of our present-day support, most of us now travel to these meetings in at least a DC-7. This latter method of transportation is more efficient but cuts heavily into the time for reflection and conversation. I hasten at this point to emphatically state, lest I certainly be labeled as ready for dejection from this Society, that I do believe that residents should receive larger stipends, that research fellows should have a technician, and that so-called principal investigators should have a secretary and probably a chart maker, and that one need not understand how to repair a flow counter in order to use it intelligently.

I should like to show Figure 1 largely for nostalgic reasons. This is the first slide that I presented at a meeting of this Society, just ten years ago. Now I perversely make it my last. This shows a wooden laboratory-manufactured flame photometer. I emphasize the adjective wooden, for just two years after this slide was first shown the instrument catastrophically and hideously caught fire and burned up. It was one of my earliest lessons that much of one's labors often quite literally go up in smoke. The circumstances leading to this debacle have probably been secretly shared by all of us. The abstract written in March with its splendid and confident conclusions turned out, as the enslaving machine turned in more data, to be all wrong. In addition, no one had really thought that the president would be intelligent enough to put the paper on the program. The day of this conflagration was Sunday, and the fatal day for the presentation was the following Tuesday. Wooden flame photometers were not designed to do over a whole experiment in seventy-two hours.

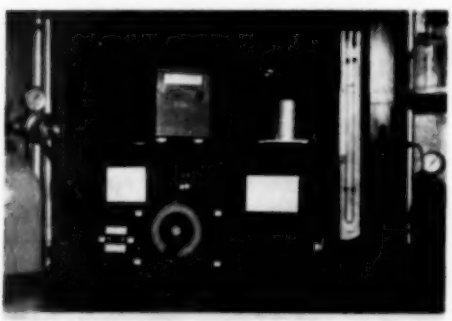

Figure 1

I speak of this episode of destruction because it taught me several lessons. One hears of much torment and anguish about the pressure to "get out a paper." All of us suffer from considerable inertia when it comes to organizing our year's work. What seems so clear in casual conversation around the coffee pot in the laboratory is, in reality, quite hazy and often represents little but poorly disguised hope that such and such is true. I would remind you of an aphorism of Dr. Darrow's: "We are all such reasonable fellows that anything could happen." The abstract deadline leads to the secretion of highly potent organizing substances. Data must be evaluated, sorted, and tested against hypothesis at periodic intervals. Certainly without the yearly stimulus of this Society our work would become an incubus of enormous magnitude, with untested piece added to untested piece in the hope that the definitive opus that leaves no argument will soon emerge. One even fondly likes to imagine an editor requesting that we take a whole issue for a series of Roman-numeraled articles of superb and definitive clarity. It never really works out this way. One should remember that good work is never finished. There is always another question to be answered. I also suspect that the ten-minute presentation time of this Society is a blessing in disguise. It forces things into the black and white. The shades of gray can be left for the hotel bar and lobby, where they belong.

The destruction of our cherished instrument by fire represented the loss of over a year's hard work devoted to method alone. I suppose that this is one of the hazards of the investigator's life. One hears much from those sophisticated in operational analysis, from those expert in the "structuring" of broad research programs, that investigators should spend little time in the development or use of technique. They tell us that these adjuncts of research should be purchased and eventually entirely automated, lest the investigator commit the sin of wasting his time in menial labor. It is often said that one should store up methodless ideas for research and wait until someone comes along with the method or instrument to do the job. I have always had a certain quarrel and little patience with this way of doing things. In the first place, the fellow who comes along with the machine may very probably beat you to your idea. It is often very hard to realize that other people think of things just as you do. Technique is analagous to the development of language in the child—without it concept and hypothesis can have no real expression or testing. Methodology is one of the most positive antidotes for the intellectual sins of oversimplification and overabstraction. Possession of techniques allows for the breaking of new ground where the gamble of finding something is low but the excitement and pleasure of the work is high. Also, while it is not nearly so much fun, one still grows the best crops in old and well-tilled soil. Here again, technique is the potent key. The search for method, for technique, brings one into contact and friendship with those in fields diverse from the pragmatic empiricism of clinical medicine. What may have started as merely a search for a new tool leads into wider areas of concept that can be brought back to enrich and sharpen your original idea. A fourth dividend can certainly be said to lie in the field of mental hygiene. One cannot sit and actively think and write papers all day long (at least I cannot). A certain amount of time spent in the laboratory calibrating a manometer engenders a productive non-forced flow of thought about one's problems. The highly efficient head of a department will probably say that it is better to go down and help in the outpatient department or see some patients. While, by the nature of my present assignment, I must be highly in favor of a certain amount of this, I would warn you privately to be judicious about it. Finally, possession of technique allows one to exploit one's non-laboratory friends. One can get in on the cases you want to study without the hard work of screening the patients. In a year or so, after the cream is skimmed off and everyone knows as much as you do, the possession of technique may become a nuisance. At this juncture it may be wise to plead that the everyday use of the method is "interfering with research" and then you may sell it to the administration to make money for the routine laboratory.

The investigator working in a clinical department is always in the somewhat ludicrous but highly effective position of having to ride two horses at one time. He is expected to achieve nonchalance and the complete appearance of outer security about Grade 1 diastolic murmurs. Additionally, he must acquire at least a moderate degree of security in nonclinical areas upon which the present-day structure of medicine is erected. He must become a reasonably competent biochemist, microbiologist, psychologist, pathologist, or physiologist. To do this takes time away from becoming expert in one's original choice of profession. One is very likely to find oneself in the difficult position of knowing that his biochemical friends regard him as an excellent doctor and a nice fellow and that his clinical friends think of him as perhaps a nice fellow but an excellent biochemist. It is the old question of fish, fowl, or good red herring. This Society has always provided a respite from such insecurities, and I hope that it will continue to do so.

A further hazard faced by the pediatric investigator is the dependent position of

the child and, its corollary, his small size. This latter hurdle has been admirably taken by the ingenuity of many members of this Society. Small size has furnished some of the bright spots in pediatric research; however, I still remain secretly a little vexed when I hear that one of my colleagues working with patients with surface areas above 1 square meter is planning to put a special catheter in such an improbable place as the adrenal vein and chromatograph a liter of blood while he simultaneously produces anxiety in the patient. I have no easy answer to the technical, legal, and special humanitarian problems that face the pediatric investigator; however, this matter of size does give me the opportunity to touch upon a subject of considerable toxicity in present-day pediatrics. Most of us in this Society are quite unregenerate organic isolationists. Acceptance of this point of view has led to the assemblage of an enormous body of facts and techniques by means of which the natural history of disease can be advantageously modified. While we have been busy with these affairs, the community has wisely seen the necessity for infiltrating our preserves with nonorganic partisans of child care. If we accept the fact that a reduced filtration rate can diminish nitrogen balance, slow physical growth, and modify the hydrogen-ion concentration of the body fluids, then it would seem only logical for us to accept the rather conclusive evidence presented by our new-found colleagues that such a deficit can prevent the orderly progress of the child toward maturity. They have sought our judgment and knowledge concerning organization of pediatric care and have offered their aid. Certainly our goal is a common one, and we have much to gain by being a little more reasonable and gracious about accepting their aid.

A further item creating anxiety in the investigator I will try to show in Figure 2. You will all recognize the questions and no Most of these demands for information are easily satisfied. I call your particular attention to Item C, which I have underlined: "Significance of this research—explain why the results of the proposed work may be important." On the surface this is quite reasonable and sensible, but there are few in this Society who would not agree that the tortured self-analysis involved in getting doubt have spent many hours of toil attempting to be convincing about the answers. this one into a printable form makes one

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RESEARCH PLAN AND SUPPORTING DATA

On the continuation pages provided give details of the proposed plan and other necessary data in accordance with the outline below. Number each page, the first continuation page being page 4. Additional continuation pages, if needed, may be requested from the Division of Research Grants. See detailed instructions before preparing this portion of the application.

1. RESEARCH PLAN
   - A. Specific Aims—Provide a concise statement of the aims of the proposed work.
   - B. Method of Procedure—Give details of your plan of attack.
   - C. Significance of this Research—Explain why the results of the proposed work may be important.
   - D. Facilities Available—Describe the general facilities at your disposal. List the *major* items of permanent equipment.
2. PREVIOUS WORK DONE ON THIS PROJECT
   Describe briefly any work you have done to date that is particularly pertinent.
3. PERSONAL PUBLICATIONS
   Cite your most important publications on this or closely related work. List no more than five.
4. RESULTS OBTAINED BY OTHERS
   Summarize pertinent results to date obtained by others on this problem, citing publications deemed pertinent. Select no more than five.
5. BIOGRAPHICAL SKETCHES
   Provide brief sketches for *All* professional personnel selected who are to be actively engaged in this project.

Figure 2

often hope that Congress will not appropriate the requisite funds. There is little point in quoting the historical evidence that points the philosophical fallacy in this request. I should say at this moment that I long ago learned that those who administer the funds that so often come in response to such requests are as aware of the problem as we are and have shown the fortitude to allow the record to speak for itself and have never coerced us into adherence to the injunction in question. Those for whom subsidy connotes socialization and control should examine these transactions between society and medical research. Could they overcome their prejudices, they would be wiser for having done so. A year ago, before this Society, Dr. Zuelzer examined in detail the subtle operations by means of which the donor can intellectually consume the recipient and spoke of the mechanisms by which we can counteract this trend. I speak of it in a more personal fashion with regard to the equinimity of the investigator. Even if we can find our way around the problems he posed, most of us at work in clinical departments are subjected to the unsettling inner and outer coercion to find the solution to the purely pragmatic question. It is never that easy. One's only consolation is to heed the words of Alexander Pope, in "An Essay on Man":

> Truths would you teach, or save a sinking land
> All fear, none aid you and few understand.

It is probably of significance as well as a good thing that one is retired from this Society at a time when one can no longer read the graduations on a burette and write in a notebook at the same time with any reasonable facility. Furthermore, granting agencies will not allow the purchase of specially marked glassware with large numbers; this comes under the category of permanent equipment. This you must either buy for yourself in some way or other or make a special trip to Washington. In the peripheral sessions of this Society one hears much discussion of the investigator's climacteric. The tone of such talk is usually such as to indicate that the discussors have a liberal, mature, and understanding attitude about some of the items appearing on the program of the senior pediatric society.

I should like to speculate for a moment about cast of mind of the successful investigator. He should have a good intellect but not one so good that he leaves principles and concepts hanging in the air. He should be possessed of inquisitiveness of mind and have enough ambition to drive his curiosity to some reasonable fruition. At the same time he should not have so much ambition that he allows none around him to share in the fun. He must have a toughness of mind that keeps him from bondage to his theories or the theories of others. He must learn to develop the ability, perhaps he must be born with it, to correlate ideas as they apply to his own particular problem. Above all, he must be possessed of a certain large and liberal discontent with things as they are.

Most of these endowments or acquisitions would seem to be fairly durable and to persist, and even enhance themselves, as the deadline for removal from this Society approaches. The collective wisdom of the group as expressed in its bylaws says otherwise, and I will not undertake to quarrel with it. There is even said to be a body of statistically valid information that reinforces the position taken by this Society. It has always seemed to me that the fading of the discontent with things as they are is the event that begins the downfall of the investigator. Santayana [1] expresses firmly and pessimistically the thought that creativity in philosophy, art, and science is characteristic only of the adolescent phase of life. He writes: "Fixity of tradition, of custom, of language is perhaps a prerequisite to complete harmony in life and mind. Variety in these matters is a lesson to the philosopher and drives him into the cold arms of reason, but it confuses the poet and the saint and embitters society." Fixity of custom, tradition, and language are all at odds with the precepts of governing accomplish-

ment of successful research. Perhaps we come too much to value complete harmony of life and mind.

I know of no good way to avoid this hazard of the investigator's existence nor am I so sure that some subtle shifting of oxygen atoms in one's adrenal or gonadal steroids accounts for the change. The rule of this Society calling for emeritus status at a given age is probably based on statistical evidence indicating that investigational obsolescence begins at this point. Too many of us take comfort in the realization that statistics have no meaning for the individual. We are always certain that only the other fellow will be affected. One should avoid this error in thinking. I can describe certain socially acceptable substitute rationalizations that are useful for easing oneself out of the investigator's role. Successful accomplishment of research is one method of entree into the complex accessory apparatus that we have constructed to administer medical practice and research during what Dr. W. Barry Wood [2] has termed its "logarithmic phase of growth." Absorption in committee meetings, administrative detail, and attendance at all manner of medical meetings or even engagement with the presently popular "team" or "platoon" techniques of research can become effective ways to insulate oneself from the tribulations of personally working in the laboratory or on the ward. They can also admirably support your ego. The mass action constant of the reaction that tends to precipitate the investigator out of solution is a very, very small number expressed with large negative exponents, and the reaction, once started, tends to go quickly and completely. The ionic concentrations and environmental conditions that tend to prevent the precipitation should be carefully considered. If one does not do this, he is quickly cut off from the contact with the laboratory and with younger men, upon whom most of our salvation depends. Without these close and concrete associations one quickly becomes incapable of even directing research at the so-called "policy" level.

At this point it would be best if I heeded the warning from Ecclesiastes: "He that diggeth a pit shall fall into it." Presidential addresses, I am afraid, can fall into the category of some of the things I have just tried to warn you of. I have tried to speak of some of the problems that beset us all and on which we are loathe to speak out loud. I realize well that I have said nothing new, that you will not all agree with me, and that I have committed the error of showing only one side of the coin in caricature. For me personally this Society has done much to resolve the conflicts that I have spoken of. For this aid and for the present great honor of being its president, I wish to thank it.

As we are about to begin the presentation of scientific papers, which must be positive things, and since I have spoken in what may be regarded as a negative manner, I would remind you at this moment of the soldier's apothegm: "Don't take counsel with your fears."

School of Medicine, Western Reserve University.

REFERENCES

1. Santayana, G.: Persons and Places, London, Charles Scribner's Sons, 1944.

2. Wood, W. B., Jr.: The Logarithmic Phase of Medical Progress: Presidential Address, The American Society for Clinical Investigation, J. Clin. Invest. 31:611, 1952.

Society Transactions

SOCIETY FOR PEDIATRIC RESEARCH

Twenty-Seventh Annual Meeting, Carmel, Calif.
June 19, 20, 21, 1957

Significance of Pulmonary Vascular Pathology in Ventricular Septal Defect as Determined by Lung Biopsy. PAUL ADAMS JR.,* RUSSELL V. LUCAS,* DONALD K. FERGUSON,* and C. WALTON LILLEHEI,* Minnesota. Introduced by JOHN A. ANDERSON.

Fifty patients undergoing intracardiac surgery for ventricular septal defect had lung biopsies at the time of surgery. Lung sections on autopsied cases have also been reviewed, many of which cases also had lung biopsies just prior to death. Elastic tissue staining was used on all sections. Biopsy and autopsy sections were graded for severity of pulmonary arteriolar change according to whether they were (1) normal, (2) Grade I (medial hypertrophy plus no more than two vessels with intimal proliferation per square centimeter), (3) Grade II (three to five vessels with intimal proliferation per square centimeter), or (4) Grade III (six or more vessels with intimal proliferation per square centimeter). The gradings were done with no knowledge of the patient's name or subsequent course in hospital.

Fourteen of the sections showed Grade III pathologic changes, and only three of these patients survived surgery. Incidentally, none of these patients showed systemic desaturation.

Fourteen of the sections were graded as normal, and all of these patients survived surgery except one.

Sections graded as I or II comprised the remaining 22 cases. Seven of these patients failed to survive, and the deaths were nearly equally distributed between the two groups.

Little correlation was shown between pulmonary pathology and direct physiological measurements of pressure, on the one hand, and calculated measurements of flow and resistance, on the other. The ECG findings were not helpful. However, when the pulmonary artery systolic pressure was plotted against age it was noted that 13 patients whose points fell into the upper right quadrant showed Grade II or III lung changes and only 4 survived surgery. This arbitrary dividing line may be expressed simply by a formula. The pulmonary artery systolic pressure plus the age of the patient multiplied by 3 was over 110 in the bad-risk patients and under 110 in the better-risk patients.

This study makes available for the first time lung tissues from patients who have survived intracardiac surgery for closure of ventricular septal defects. Although many other factors undoubtedly affect immediate and prolonged prognosis after surgery, in the present state of our knowledge pulmonary vascular pathology appears to be the most important single item. It is felt that the "margin for error" is diminished in the face of severe pulmonary changes graded as II or III. In patients showing values by the formula stated of more than 110, lung biopsy may be justifiably considered as a preliminary procedure to more quickly validate this relationship and to give added information for deferral of surgery or use of procedures designed to combat pulmonary complications.

Discussion

HOWARD A. JOOS, Los Angeles: Dr. Adams and his co-workers are to be complimented on an elegant study. I wonder if he related his histologic observations to the ability of the pulmonary vascular bed to respond flexibly to stimuli such as low-oxygen inhalation and exercise. The adaptability or rigidity of pulmonary vascular resistance may reflect the physiologic derangement implied in the anatomic observations more profoundly than resistance or pressure measured only under static resting conditions.

PAUL ADAMS JR., Minneapolis: Dr. Joos, we did not study the factors that you mention. You will notice that many of these patients were very young and of course were not susceptible to exercising studies, such as were very nicely presented in the paper this morning. I think that that certainly might help us further to delineate the poor-risk type of patient.

The Syndrome of Addison's Disease, Hypoparathyroidism, and Superficial Moniliasis. ANGELO M. DIGEORGE* and KARL PASCHKIS,* Philadelphia. Introduced by VICTOR C. VAUGHAN.

The coincidence of Addison's disease, hypoparathyroidism, and superficial moniliasis in more than one sibling has been reported in only three instances. However, sporadic cases of (1) hypo-

* By invitation.

parathyroidism and Addison's disease, with or without superficial moniliasis; (2) sporadic cases of idiopathic hypoparathyroidism associated with chronic moniliasis; (3) familial cases of hypoparathyroidism; (4) familial cases of Addison's disease, and (5) instances of Addison's disease in one sibling and idiopathic hypoparathyroidism in another sibling have all been reported.

This report deals with the fourth family with all components of this syndrome. Of four siblings, one is normal at 8 years of age and one died at 5½ years of age of Addison's disease. Another child developed Addison's disease at 8½ years of age and hypoparathyroidism at 10½ years of age. The last child developed a celiac-like syndrome at 2 years of age, superficial moniliasis at 3 years of age, and hypoparathyroidism at 6½ years of age.

Detailed chemical and endocrinologic studies on the last two children will be presented. Normal serum calcium and phosphorus levels were present two years and nine months, respectively, prior to the development of hypoparathyroidism in the two affected children.

Particular attention will be given to the celiac-like syndrome and to the parathyroid-adrenal interaction in this syndrome. It is suggested that all of the above disorders represent variable expressions of the same hereditary trait.

Discussion

ROBERT KLEIN, Pittsburgh: I think this is very important, because Dr. DiGeorge really gives the outline of the first case that I know of in which the patient had Addison's disease before the hypoparathyroidism. The hooker in the past cases has been that the advent of Addison's disease always makes the hypoparathyroidism better.

If you give cortisone you increase the secretion of calcium. This, incidentally, makes the Sulkowitch test unreliable, but in the absence of adrenal steroids you get the reverse effect. In addition, cortisone in these patients, but not in normal persons, causes an increased reabsorbtion of phosphorus, and so when you treat the Addison disease the hypoparathryoidism gets worse. In the past cases that have been reported with Addison's disease, first I questioned whether they did not already have the hypoparathyroidism, which was obscurbed by Addison's disease.

I have a question and one statement to confuse things, and that is that the celiac-like syndrome seems to get better with the treatment of the hypoparathyroidism. I do not think that is our experience alone; other people have had it, and I can't explain this, except that as calcium increases the stools become normal.

The question I have is this: Has anybody unequivocal evidence that the sexual development of these patients is retarded or abnormal?

ARILD E. HANSEN, Galveston, Texas: It happened to be my pleasure to study one of these children with Dr. McQuarrie and Dr. Ziegler with hypoparathyroidism. We carried on rather extensive mineral balance studies and followed him for a period of some years. After he got to be past the pediatric age group, we usurped our rights, and held on to him by permission of the department of medicine. When he came in at the age of about 19, I think it was, with outspoken evidence of Addison's disease, every one of his signs of hypoparathyroidism, that is, clinical signs, had disappeared. We were going to bring him to the hospital at that time. It happened to be the day or two before Christmas, and he wanted to know if he could go home for Christmas, and we let him, and he died. We spent Christmas driving up 200 miles in the country to get an autopsy on this patient.

My question is this: Do the signs characteristically, that is, the signs of hypoparathyroidism, disappear when the outspoken evidence of Addison's disease develops?

LAWSON WILKINS, Baltimore: I was very much interested in Dr. DiGeorge's report, as we have observed several patients with hypoparathyroidism who had the celiac-like type of symptoms, and, as Dr. Klein has mentioned, in these cases the diarrhea remained completely uncontrolled by any regimen until the hypocalcemia was corrected and in periods of remission, when the calcium again fell, celiac-like symptoms appeared.

Our patient, also, and this was a patient who did not have Addison's disease, these two patients were ones who did not have Addison's disease. Now, our patient had many of the symptoms that were mentioned here today; the celiac-like symptoms lasting many years, the moniliasis beginning from a very early age, the photophobia, and the parietitis, and then at the age of 12 years and during the early periods of her life there were also intestinal absorptive deficiencies, and so forth, but trypsin was present. Later on, at the age of 12, this child developed pernicious anemia.

I should like to ask whether moniliasis has been observed to occur in hypoparathyroidism developing postoperatively as well as in the idiopathic type, because certainly the association of these things has been most remarkable and one does not know what is cause and what is effect.

ROBERT A. ULSTROM, Minneapolis: We have studied one child in addition to the patients reviewed by Dr. DiGeorge. This girl had hypoparathyroidism and disseminated Monilia infection but normal adrenals. Her sibling had the same disease and died at a relatively early age in a tetanic episode. The second child has recently died, with renal failure, perhaps due to excessive vitamin D therapy.

At the present time, we have under observation in our hospital a 3-year-old child who has had the

celiac syndrome and who has definite moniliasis. She has no evidence of endocrine abnormality that we can detect. I should like to ask Dr. DiGeorge whether he feels that a patient such as this one is a likely candidate for the endocrine abnormalities and whether he feels that the diffuse moniliasis has any direct bearing on causation of the abnormalities of the endocrine glands.

ROBERT H. PARROTT, Washington, D. C.: In the family of five children with the multiple endocrinopathy syndrome studied by several of us in Washington, the oldest affected child was a boy who died of Addison's disease and had an extensive cytotoxic dissolution of the adrenal cortex such as is sometimes seen following tuberculosis or histoplasmosis. Because of this, the rest of the family has been studied extensively for infection with histoplasmosis or tuberculosis. Two of the other children, who are otherwise well, have serologic or skin-test evidence of these illnesses; one of them reacts to histoplasmin and the other to tuberculin; both show healed pulmonary lesions. None of the other children who have an endocrinopathy react to histoplasmin or to tuberculin. My first question is this: Has Dr. DiGeorge examined the possibility that tuberculosis or histoplasmosis play a role in his patients?

ALLAN M. BUTLER, Boston: I should like to ask a question to see whether my simple thinking should be corrected. We have supposed that what has been described here as a celiac-like factor in hypoparathyroidism was due to the increased peristalsis that accompanies the low serum calcium. Indeed, we find that some older patients can make a pretty good appraisal of their vitamin D therapy from the frequency of the bowel movements. We have recently seen a patient with moniliasis who had hypo-γ-globulinemia. I wish Dr. Gitlin were here to comment, as I think he has a large series of patients who have moniliasis with hypo-γ-globulinemia.

ANGELO M. DIGEORGE, Philadelphia: Dr. Klein asked about retardation of sexual development. It is unfortunate that so many of these patients have died in early childhood. However, Whitaker's recent report concerned a child of about 15 or 16 years of age, and she did think that the testes in that child were unusually small and immature. It will be interesting to follow some of these patients over a period of years to see how many other disturbances they develop.

I do not think there is any question that the celiac-like symptoms when associated with hypoparathyroidism often improve when the serum calcium has been corrected. Many observers have reported similar observations. However, we can not explain our observation of these symptoms in the presence of normal calcium levels. Nor can we explain Dr. Ulstrom's case—the patient with celiac disease and moniliasis who does not have hypocalcemia. Collins-Williams, I believe, reported the cases of two siblings with hypoparathyroidism, one of whom had celiac-like symptoms for many years before hypocalcemia finally appeared. These are instances in which calcium levels have been determined and found to be normal in the presence of celiac-like symptoms.

Does the Chvostek sign disappear with the onset of Addison's disease? Unfortunately, in many of the instances that have been reported the onsets of hypoparathyroidism and Addison's disease occurred closely together and they were simultaneously diagnosed. In the few patients that have been reported it is difficult to draw conclusions on this point, although I agree with Dr. Klein that one would expect that the symptoms of hypoparathyroidism would improve with the onset of Addison's disease. It is known that patients with only Addison's disease tend to develop elevations of serum calcium levels during periods of crises. Experimental work done on dogs and cats has demonstrated relief of parathyroid tetany following adrenalectomy.

Dr. Wilkins asked if moniliasis has ever been associated with postoperative hypoparathyroidism. I have been unable to find any report of a patient with postoperative hypoparathyroidism who has developed moniliasis.

Dr. Butler told us about the interesting association of moniliasis and hypo-γ-globulinemia. Both our patients have normal γ-globulin levels. In answer to Dr. Parrott, we have not done any extensive studies of the serum proteins of these patients.

Comparison of Gastric Lavage and Induced Emesis in Experimental Salicylate Poisoning.

FRANKLIN J. ARNOLD JR.,* JOHN B. HODGES JR.,* SAMUEL SPECTOR, IRVING SUNSHINE,* and RALPH J. WEDGWOOD, Western Reserve.

While gastric lavage is the generally accepted treatment for poisoning, there are no available studies to prove its efficacy. Studies were therefore carried out to evaluate the procedure and to compare it with emesis in experimental poisoning with sodium salicylate.

Fasting dogs were given sodium salicylate in tablet form, and lavage was performed or emesis induced at varying intervals following ingestion. Salicylate recovery was determined in the lavage and emesis fluids. In addition, blood salicylate levels were determined.

The data indicate the following: 1. Initial aspiration, before introduction of fluid, is the most important part of the lavage procedure. The efficacy of succeeding washes will be presented. 2. Lavage carried out within 15 minutes was no more effective than emesis induced within 30 minutes after inges-

* By invitation.

tion. 3. Lavage carried out one hour after ingestion (the minimum time within which patients usually come to the hospital) was inefficient and far less effective than emesis produced within the same time interval. 4. Emesis, either immediately or delayed as long as one and one-half hours after ingestion, appeared to be effective and the preferential form of therapy.

Discussion

HARRY F. DIETRICH, Beverly Hills, Calif.: I am sure that we all are aware of the need to be very careful in transferring results from experiments with dogs to children, but to me this has been a very exciting presentation. It has been our practice in the past when parents have called us about acetylsalicylic acid ingestion to tell them to try to get the child to vomit but to not spend more than five minutes on that procedure and then to get the child in a car and take them to have their stomachs lavaged. While I should certainly like to see more of this same work and have someone else try it on the children, I think that I will have a little more confidence in trying to get patients to have their children vomit instead of immediately dashing off for the emergency hospital or the office to have their stomachs lavaged. I am sure that all of us are aware of the magnitude of this problem, but I should like to give one little fact about our particular village of Los Angeles. In one of our emergency hospitals 3000 children's stomachs are lavaged each year and over 25% of these treatments are for acetylsalicylic acid ingestion; so that I think that perhaps the authors have opened the way to sparing a good many children a lot of both psychic and physical trauma.

ROBERT J. MCKAY, Burlington, Vt.: I should like to ask Dr. Spector if he has any results of experiments in which a liquid was placed in the stomach rather than a solid and, secondly, if he has any results from instances when the stomach was not empty at the time of ingestion.

SAMUEL SPECTOR, Cleveland: I want to thank Dr. Dietrich for his comments. I might add that in our clinic we have abandoned lavage and substituted emesis for it. The emesis is induced by a large initial dose of ipecac syrup. As yet, we do not have enough analysis of the emesis fluid to provide a report of our findings in children. However, I should like to reemphasize the fact that, even under the most optimal conditions of either lavage or emesis, results are so variable that little security can be gained from the procedure and that all patients deserve continued observation and possibly further therapy.

In answer to the questions raised by Dr. McKay, I should like to state that we did not do any studies with a liquid preparation. However, the sodium salicylate rapidly went into solution upon reaching the stomach, as was evident by the fact that in both the aspirated and the vomited material the drug was recovered from the filtered fluid and was present in insignificant amounts in the solid material. The dogs were in the fasting state, and as yet we have not studied recovery rates in situations where the dogs have been fed before the ingestion of salicylate. We realize that there are many variables that need to be studied.

Studies of the Requirement for Protein in Infancy. SAMUEL J. FOMON and CHARLES D. MAY, Iowa.

Normal infants are known to thrive while receiving widely varying intakes of protein. If the protein of cow's milk were as efficiently utilized by the infant as that of human milk, the requirement for protein would presumably be the same for the breast-fed and the artificially fed infant. Because of the possibility that the protein of human milk may be qualitatively superior to that of other proteins in infant nutrition, the National Research Council has recommended an intake of protein of 3.5 gm. per kilogram per day during the first year of life for the infant receiving an artificial feeding, although the intake of protein of the infant fed at the breast may be less than 2 gm. per kilogram per day.

The present report concerns the results of feeding a preparation of cow's milk (Formula S) ad libitum, providing an amount of protein generally less than 3.5 gm. per kilogram per day but more than that obtained by infants fed human milk ad libitum. The mean intake of nitrogen of infants fed Formula S was about 505 mg. per kilogram per day at 1 month of age and 367 mg. per kilogram per day by 6 months of age, corresponding to intakes of protein of 3.2 and 2.3 gm. per kilogram per day, respectively.

The rates of growth of the infants fed Formula S were within normal limits. The mean retention of nitrogen gradually decreased from a value of 190 mg. per kilogram per day at 1 month of age to 85 mg. per kilogram per day by 6 months of age. These mean retentions of nitrogen are somewhat greater than those of infants fed human milk in a study carried out by us (170 mg. per kilogram per day at 1 month of age and 50 mg. per kilogram per day by 6 months of age).

Accepting the metabolic performance of the infant fed human milk as a standard of reference, it would seem that the requirement of the infant for protein is certainly not greater than the amount ingested by the infants fed Formula S—i. e., 3.2 gm. per kilogram per day at 1 month of age and 2.3 gm. per kilogram per day at 6 months of age. Investigations are under way to ascertain whether the intake of protein from cow's milk can be reduced to the amount supplied by human

milk without causing the retention of nitrogen to decrease below that of infants fed human milk.

Comparison of Prematures Fed High- and Low-Protein Diets. LEWIS A. BARNESS, DONALD A. CORNELY,* AREE VALYASEVI,* and PAUL GYORGY, Pennsylvania.

A better definition of minimal nitrogen requirements and optimal and maximal nitrogen allowances is necessary. In a search for optimal nitrogen allowances, humans with the most rapid growth potential, viz., premature infants, were fed two feeding mixtures essentially similar except for their protein content. Both mixtures are commonly used in infant feeding. The low-protein mixture contained 1.5% protein; the high-protein mixture, 4.1% protein. Random urine and blood samples were collected at certain weights of the infants. The infants were compared regarding total intake; rate of weight gain; morbidity; mortality; total serum proteins with protein fractionation; hemoglobin; hematocrit; blood urea nitrogen; nitrogen excretion, including urea, ammonia, amino acids, and creatinine; phenol excretion, and stool pH. A few short balance studies were included.

Most marked differences were noted in the total serum protein, especially during the first 40 days of life; blood urea nitrogen; nitrogen and phenol excretion, and stool pH. Serum proteins were higher and blood urea nitrgen, nitrogen excretion, and phenol excretion and stool pH were lower in those infants fed the low-protein diet. Rate of weight gain was slightly more rapid in the infants fed the higher-protein formula.

Details and possible significance of these results will be discussed.

Discussion

CHARLES U. LOWE, Buffalo: I wonder if it would not be appropriate to ask the authors whether they really meant that they are trying to determine the minimal protein requirements of infants. It seems to me what both of them have demonstrated is that, when a diet is constituted as they have described, infants may grow well on different protein intakes. Entirely forgotten in this discussion is the effect of calories. I think that any program designed to determine the minimal nitrogen requirements for infants must do so while at the same time varying the caloric intake. Now, I do not believe that either of the authors would take exception to this, but I wonder if they would not like to add to their paper the fact that the caloric problem has not been evaluated.

We have been recently attempting to correlate the information which is available on the relationship between protein intake and nitrogen retention while the calories are varied, and it is possible to construct a diagram with three variables which indicates that for any protein intake, if that is within certain limits, a caloric intake exists which will produce a specific or anticipated nitrogen retention. This means, of course, that nitrogen requirement can only be examined in relation to total caloric intake.

HARRY H. GORDON, Baltimore: One of the things that concerns me has been referred to by Dr. Lowe. It has to do with the attempts to conduct balance studies in the current psychological era of infant feeding. There was a time when we all believed that a proper balance study required a foreperiod of carefully controlled intake for at least three or four days before one started collection of urine or stools. In the paper presented by Dr. Fomon the intake is described as ad libitum for an indefinite period with metabolic balance studies made for three days every two weeks, and I can not tell whether intakes are ad libitum in Dr. Barness' study.

With respect to one of the criteria of the adequacy of these feedings, we are told by Dr. Fomon that the weight gains were within normal limits, but no comparisons of weight gains were made.

Dr. Barness mentioned a difference in mortality, which he said he does not think is important but which he mentioned twice. I should be interested in the causes of death. For the most part, our methods of infant feeding do not kill babies, whether the diets are high or low in protein, unless the infants are permitted to choke.

ALLAN M. BUTLER, Boston: I shall not attempt to be as concise as I was in commenting on Dr. DiGeorge's paper, because in my conciseness there was perhaps a misinterpretation of intent in quoting some observations of Dr. Gitlin. I did not wish to impress the audience with the number of patients seen in Boston, but rather to refer to some observations made on a relatively few patients, so that when one had a patient with moniliasis one would think of hypo-γ-globulinemia as well as Addison's disease and hypoparathyroidism.

The reason I now speak is not only to say how significant these two papers are in providing a sound reorientation to formula feeding of protein per kilogram of body weight but also to emphasize the need of considering protein requirement in terms of the age of the infant.

In the recommendations of the National Research Council on desirable nutritional allowances you notice that there are no quantitative data given for the infant under one month of age; there is merely the statement that in feeding infants under one month of age daily allowances should conform to the maturation of the infant's

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renal and endocrine homeostatic capacities. From the curves on the charts of the first paper one might be led to conclude that infants under 2 weeks of age consumed more grams of protein per kilogram of body weight than older infants. That may be so in these experiments, where babies were presented with a certain volume of breast milk in a bottle, but, I take it, in nature such amounts of breast milk are not available immediately. For the baby under 2 or 3 weeks of age the desirable amount of protein per kilogram of body weight may more nearly approximate what the breast-fed baby really gets, which is even lower than the figures presented today.

SAMUEL Z. LEVINE, New York: I think it is important to point out what was pointed out before, that we do not know how much nitrogen should be retained in the body at any age period. Certainly with increased protein intakes there is an increased nitrogen retention. Now, Dr. Barness' mentioned in his paper that the premature infant gained more when he was on the high-protein diet than on the low-protein diet; the difference, I think, is 2 gm. per day, roughly. The period of study was 21 days. That would be a gain of 42 gm., total gain. In Dr. Fomon's paper there was no mention of the rate of weight gain in either group, but in both weight gains were satisfactory. Now, if the total weight gain were even somewhat greater on the high-protein intake one would expect that the total water, whether it be related to ash or not, would be higher and that the total weight might require a greater nitrogen retention. Furthermore, I think that if one calculates Dr. Fomon's data in terms of nitrogen retention per unit of weight gain at the different ages, at 1 month of age and at 6 months of age, one will not find these large discrepancies between the concentration of nitrogen and weight. Another point that I think ought to be emphasized is that when we are dealing with a premature infant the natural food in utero is certainly not milk, it is blood, and the protein content of blood is different from the protein content of milk. Therefore, the premature infant may require an entirely different amount and quality of protein than the full-term infant. Again, if one assumes that the optimal weight gain of the premature infant postnatally should simulate the weight gain if the baby had remained in utero, the total weight gain per day is around 25 gm. per day. In this study it was 19 gm. If 25 gm. were attained per day, one might assume that a higher nitrogen retention might be preferable to the amount of nitrogen retained with the lower protein intakes.

BENJAMIN M. KAGAN, Los Angles: Drs. Barness, Gyorgy, and their co-workers noted lowering of the hemoglobin, hemotocrit level, and serum protein values with higher ash intake. They suggest that this may be explained as due to possible "intravascular hydration." This would be in accord with interpretation of our previous related studies.

We are presently measuring total body water and extracellular water in premature infants before and after isocaloric feeding from the 7th to 28th days of life. The infants are being fed human milk, human milk plus added minerals, or a high-protein, high-ash, low-fat cow's milk. The total difference in body water over the 21-day period in infants weighing about 1500 gm. may be only about 180 ml. Water-balance studies based on collections of stool, urine, and sweat might not reliably pick up retentions of water of the magnitude of only 3 to 5 ml. per kilogram of body weight per day.

Along with the previous commentators, I should like to know whether the feedings were isocaloric and, if so, at what rate.

With accumulation of such valuable information as that presented in these two papers, we can reasonably hope that we will soon graduate from the "descriptive phase of infant nutrition," as it was so aptly put by Drs. Fomon and May.

L. EMMETT HOLT JR., New York: Dr. Snyderman and I have obtained some data in many ways comparable to that presented today on low-protein intakes both in premature infants and in full-term infants. We have attempted to determine not the spontaneous intakes but minimal intakes compatible with health. The figures we obtained are considerably below those in the two papers given today. The real question, which cannot be answered yet, concerns the optimal and not the minimal requirement for premature and full-term babies. All we can say is that extremely low intakes, much lower than we have been led to believe, are compatible with normal growth, with adequate nitrogen retention, with the maintenance of health, and with no more evidence of infection than other children under comparable conditions seem to have. The question boils down to the value of reserve protein. There is, as is well established, a direct relationship between nitrogen intake and nitrogen retention. In part it is a temporary phenomenon. The body adapts itself to the higher intake and after a while it excretes more nitrogen. But after that period of adaptation is made, there remains a slight difference in body composition. This was demonstrated a number of years ago by Osborn, Mendel, Winternitz and Park (*J. Biol. Chem.* 71:317, 1927), the chief differences being in the weight of the kidney and of the liver. Animals on a high-protein intake need more kidney to excrete urea, and they need more liver for purposes of deamination on a high-protein diet. Presumably this holds for babies, too. A large part of the reserve protein is located in those two organs. Is a baby or animal with such reserves in a better condition to withstand

various stresses that may be encountered? That is the question I think we need to have more information about.

SAMUEL J. FOMON, Iowa City: Formula S supplied approximately 20 calories per ounce. We presume that the human milk pool from a relatively large number of donors, usually not less than 18 donors on any one day, also supplied approximately 20 calories per ounce.

Let me clarify what was meant by ad libitum feeding. The babies were offered the largest amount of food which they seemed to accept consistently, and adjustments in the volume of feeding were made only on the day after a balance period was completed. There was thus in every case a period of at least 10 days that the infant received constant volume of intake before a balance period was carried out. Ad libitum feedings with this slight modification were employed in both studies. In the case of Formula S the concentration of nitrogen in the feeding was constant throughout the study. In the case of human milk it varied somewhat, depending on differences between groups of donors contributing to the collection center.

The point concerning gain in weight I will emphasize again, as I have previously. We believe that conclusions drawn from a comparison of gains in weight between such small groups of infants are likely to be misleading because of factors of individual variability. Such comparison should be based on studies of larger groups of infants.

LEWIS A. BARNESS, Philadelphia: I should like to start by commenting on Dr. Holt's comments, which I really appreciate. I want to say that the study which was presented here today was not, Dr. Holt, to try to find the minimum nitrogen requirement at which babies would thrive. This study, as I indicated in the introduction, was simply one of the by-products of a study of minimal nitrogen allowances. Actually, when we first started studying minimal protein concentrations upon which babies could thrive, we came across an article by Dr. Gamble, written in 1911, and I spoke to him at one of these meetings and told him that we were again doing what he had done then. He assured me that much work had been done at that time on minimal protein requirements without an answer and that it would probably be many years before anyone did come to an answer.

As far as the body composition differences are concerned, Dr. Holt, this has troubled us a great deal too and has probably troubled us more since Dr. Wallace's paper, published last year, in which he showed that regardless of protein intake there was very little difference in the nitrogen composition of various pertinent tissues.

[Slide] I should like to point out that the concentrations of calories were not exactly the same in these two formulas, but they were very close. Dr. Lowe, we were trying to feed a diet adequate for growth. It was 69 and 67 Cal. per 100 cc. The actual caloric intake of these babies was between 120 and 140 Cal. per kilogram during the study.

As far as the balance studies that we did do, I should like to point out again that none of the data we presented here today were obtained by balance techniques except for some of the phenol measurements. The other measurements were all concentrations with timed specimens of urine. I think Dr. Kagan has answered you regarding our feeling about the water balances, Dr. Gordon; so I won't belabor that.

The causes of death were as follows: seven died of infection; one, of hyaline membrane; two, of congenital anomalies, and one, of undetermined cause. The reason I repeated this twice was not Freudian, and I had no vile purpose in it except that these data were not included in the abstract.

Dr. Butler, we also have been trying to find out about the feedings in the first few days and have gotten into too many technical difficulties. We agree that an early high-protein intake may very easily overload the immature kidney.

Dr. Levine has posed the one problem that really confuses us, and that is why babies gain 25 gm. a day in utero and only 17 or 19 gm. a day out of utero. We don't know why this is or if this is significant.

An Epidemic of Febrile Pharyngitis: ? Example of Recurrent Infection with Adenovirus. DAVID H. CARVER,* ELI GOLD,* and FREDERICK C. ROBBINS, Western Reserve.

In August, 1956, an epidemic of nonstreptococcal febrile pharyngitis occurred among the preschool children at a Cleveland orphanage. During a four-day period 17 of the 49 children in the unit became ill with an illness characterized by fever, cervical adenopathy, pharyngeal inflammation, and exudate. All were admitted to the infirmary.

Stool, blood, and throat specimens were obtained from all inhabitants of the cottage, whether ill or well. Beta-hemolytic streptococci were not cultured from the pharynges of any of the persons. Throat and stool specimens were inoculated into HeLa and MKC tissue cultures and passed at least once.

Type 1 adenovirus was isolated from five persons, four of whom were in the infirmary with illnesses. The fifth isolation was from a child considered well but noted to have an exudative pharyngitis at the time the culture was taken. The serum of all persons was tested for the

* By invitation.

presence of neutralizing antibody (AB) to Type 1 adenovirus. The results were as follows:

1. Sixty-six per cent of all children possessed neutralizing AB against the Type 1 adenovirus at the start of the study.

2. There was no relationship between the presence of Type 1 AB and illness; 12 of 29 children with AB became ill; 5 of 15 without AB became ill.

3. Four of the isolations of Type 1 adenovirus were from ill patients who had no AB when they became ill. The fifth was from a well patient with no AB in the initial serum specimen.

4. Convalescent sera were obtained from four of five of the patients from whom adenovirus Type 1 was isolated. All showed a fourfold or greater rise in AB.

5. No viral agents could be isolated from the throats or stools of the 12 persons who had Type 1 AB when they became ill.

6. No increase in adenovirus Type 1 AB occurred in the 12 persons who had AB when they became ill.

To evaluate the etiological importance of the Type 1 adenovirus in the illness of these 17 children the following points were considered.

1. All illnesses began within a four-day period and were clinically similar; this suggests a similar etiology.

2. The Type 1 adenovirus was isolated from all sick persons who possessed no antibody at the time they became ill. All tested had antibody in a convalescent serum specimen.

3. The adenovirus was isolated only from sick persons. If it were a nonpathogenic agent coincidently occurring in the population it should be present in the well children as frequently as in the sick.

4. The adenoviruses are known to cause febrile pharyngitis.

If it is assumed that all 17 children suffered from an adenovirus Type 1 infection, it would then follow that the patients who had antibody when they became ill experienced a recurrent Type 1 adenovirus infection. It would seem that the presence of antibody in their throat or stool specimens made detection of virus impossible with available techniques. Other observations supporting the possibility of recurrent adenovirus infections and implications of this concept will be discussed.

Discussion

C. Henry Kempe, Denver: I think the routine utilization of virus techniques is getting to be very similar to the use of those of bacteriology in the last 30 years.

The very point of this paper is the very point that worries me. First of all, the presence of virus in the face of massive antibodies is common, of course, in the case of herpes simplex infections with huge titers. In influenza, where a situation very similar to the one Dr. Gold has described should hold, we also have trouble isolating the virus in the face of the antibodies. The first question I want to ask is whether the authors have tried to dilute out the antibody by making dilutions of the throat-washing material in an effort to isolate the virus. This technique is widely used for other respiratory viruses. This would be one way to try to get a virus in spite of antibodies in the sputum. Now, the thing that worries me about the 14 children who have antibodies and have illness is this: If the virus is able to destroy tissue and destroy cells, then a cytopathic effect should also occur in tissue culture. We say that if a diagnostic antibody rise occurs in serum then an antigenic experience has occurred. Now, these 14 children have not had this antigenic experience documented; there is no rise in antibodies. Why do they not rise? One explanation might be that the children do not have this particular virus. In fact, as not a single isolation was made, it would seem possible that in fact none of these children were suffering from the disease in question but rather that an altogether different, as yet unisolated, agent caused the entire epidemic.

Robert H. Parrott, Washington, D. C.: In the past the criteria for demonstrating infection with a virus have included either isolation of the virus or evidence of a rise in antibody against the virus. Under these criteria there is no direct evidence presented in this paper for infection with Type 1 adenovirus in the 14 children who had antibody previously.

I wonder whether the level at which the antibody tests were run could have been of significance in settling the problem here. It may be of interest in this regard that two older persons in the first reported ward outbreak of pharyngoconjunctival fever had minimal complement-fixing antibody titers previous to the outbreak; in both, however, either virus was isolated or an antibody rise was shown concurrently with illness.

Heinz F. Eichenwald, New York: Like the other discussers, I think the points that have been brought out in this paper are extremely interesting and provocative. Perhaps one should consider other possibilities to explain these findings.

The epidemic curve of this particular episode suggests that this was a common-source outbreak, and this brings me to the first question: Was it possible to identify the source? If it was a common-source outbreak, it should also be possible that at the time the Type 1 adenovirus was introduced another agent, not identifiable by present methods of tissue culture, was introduced, so that the children, in fact, were suffering from a double infection, only one agent of which was isolatable.

We have been interested in adenovirus infections in small infants and have studied a few cases where infants who had acquired Type 1 antibodies passively from their mothers acquired Type 1 adenovirus infection during early infancy. It was rather easy to isolate the virus from the nasopharynges of these cases. And this is my third question: Has it been possible to show that the pharyngeal secretions of these children contain viral inhibitory substances or antibodies?

ELI GOLD, Cleveland: Dr. Kempe, regarding methods for detecting virus by dilution of antibody, we did try such techniques. Actually, we tried so hard to isolate virus from the throat washings before we had information about the antibody status of the patients that no specimen remained for the dilution methods, but we did have stool specimens from all persons. Suspensions of stool were adsorbed to HeLa, monkey, or amniotic cells for four hours, and then the tubes were washed and new maintenance fluid was replaced. We did isolate virus by this technique, but only from the persons from whom we had obtained virus from the throat. None of these possessed antibody when they became ill. None of the tricks we were able to try resulted in isolations in patients who had antibody to Type 1 adenovirus when they became ill.

As to the evidences of rise in antibody, we tried neutralization tests with various amounts of virus with no success. It occurred to me that perhaps the complement-fixation titers might have fallen more than the neutralizing titers and that we might be able to demonstrate a booster effect by the rise in the CF, even though we could not do it by neutralization. The CF results, however, were similar to the neutralization results, and we were not able to show any rise in antibody in our patients, all of whom had high initial levels of antibody. This type of observation has been recorded with recurrent herpes simplex, for example.

Dr. Parrott, the ordinary criteria for evidence of infection by a virus are as you stated, and perhaps there is some reason to change these criteria. Our conclusions are hypotheses. They seem to be something which the data suggest and something which I thought would be interesting to talk about.

Dr. Eichenwald, we tried to find the source, and the best answer we have is the fact that visiting day at this particular orphanage was seven days prior to the onset of the first illness, but we were not able to find any particular person with an illness.

As for your suggestion that perhaps there was a double infection—that there were two viruses here, one of which we could not detect—I think this is a likely possibility, but, again, the fact that the Type 1 adenovirus was demonstrated only in sick persons and that the epidemic had a single peak makes me feel that the virus had some relationship to the illness.

The Nonspecific Inflammatory Response in the Newborn Period. DONALD V. EITZMAN,* and RICHARD T. SMITH, Minneapolis.

Compared with older infants and adults, the newborn infant has been shown to have decreased skin reactivity to diverse irritants, such as Schick and Dick toxins, croton oil, or eel serum, and to certain infectious agents. In seeking an explanation for this apparent deficiency, the sequence of early cellular changes occurring during nonspecific inflammation was examined in a series of 50 infants, 10 hours to 9 months of age. For these studies a modification of Rebuck's technique of serial coverslip imprints was employed, with sampling at 2, 3, 4, 6, 8, 10, and 12 hours following a standard 1×1 cm. abrasion, and 200- to 500-cell differential counts were made of each stained preparation.

Between one and two hours the exudate consisted almost entirely of polymorphonuclear granulocytes with occasional mononuclear cells. At three hours hematogenous macrophages began to appear, and by 6 to 8 hours they comprised 30% to 60% of the inflammatory cells. However, in all but one of the groups of normal infants of 36 hours to 21 days of age, from 10% to 93% of the cells in the one- to three-hour exudates consisted of eosinophils. Maximal eosinophil content was usually found two hours after initiation of the cycle. The highest proportion of eosinophils was found in the exudates of infants with high circulating eosinophil counts, but in all infants in this age group inflammatory eosinophils were present in the exudates much out of proportion to circulating levels. In contrast, no normal infant less than 36 hours of age or more than 21 days of age exhibited this phenomenon, nor did the older children and adults studied.

Serial examination of the early inflammatory cycle in several infants, beginning shortly after birth, has confirmed the appearance of inflammatory eosinophilia after the second day of life.

With the exception of the transient appearance of eosinophils, the sequence of early inflammation in the neonatal infant was not qualitatively different from that of older children. However, older children and adults had a significantly higher percentage of mononuclear cells in 6- to 10-hour exudates than the young infants.

While the observed inflammatory eosinophilia is not understood, its occurrence suggests that the eosinophil content of the lesions of erythema neonatorum is entirely nonspecific with respect to the inciting irritant. Further studies of the later phases of the inflammatory cycle in infants are in progress.

With use of the connective tissue spread technique of Kolouch, the serial morphologic changes in

* By invitation.

nonspecific inflammation in newborn rabbits have also been investigated. The data available thus far suggest that the sequence of cellular changes is qualitatively similar in newborn and older rabbits. No transient eosinophilia in the early exudates was observed.

Discussion

ALFRED FLORMAN, Manhasset, L. I., N. Y.: I want to ask one question: Did the authors attempt to make an association between those babies who had erythema toxicum and those who had the highest eosinophil counts in their exudate? I ask this because, as you know, Dr. Schick pointed out sometime ago that the exudate from erythema toxicum lesions was almost pure eosinophils.

BELA SCHICK, New York: I am interested in this paper because two years ago I published a study of a skin eruption in the newborn starting about 24 hours after birth either with erythematous blotches or, sometimes, with outright hives. Two days later vesicles appear which fill with pus. This pus consists entirely of eosinophils. Seven to ten per cent of newborn infants develop this eruption. No bacteria are found. The eruption disappears without treatment. It is a toxic eruption, apparently on allergic basis. This paper was published with Drs. Gall and Harris, from the Beth-El Hospital in Brooklyn.

I think that maternal protein substances transmitted to the child react with antibodies originating in the child, producing an allergic reaction. Mayerhofer (Zagreb) published similar observations in an article about allergy in the newborn.

DONALD V. EITZMAN, Minneapolis: Thank you, Dr. Schick. We were very interested in your article and had also noted the eosinophils in the lesions. We were interested in the fact that the highest percentage of the lesions in erythema toxicum are seen after 24 hours of age, at 48 and 72 hours, when we are beginning to also see the increase of the eosinophilia in the inflammatory exudate as we follow these children along, doing serial inflammatory studies.

The results of our experiment would indicate that an eosinophilic exudate in this particular age group does not mean allergy but is the response of the infant to nonspecific inflammation.

Phagocytosis in Premature Infants. LOUIS GLUCK* and WILLIAM A. SILVERMAN, Columbia.

Information about natural mechanisms of defense against infection in premature infants is lacking, despite the failure of γ-globulin or prophylactic antibiotics to effect an appreciable decrease in incidence of infection. The present study deals with one phase of defense against infection, phagocytosis.

Phagocytosis in 54 premature infants ranging in birth weight from 630 to 2200 gm. was studied on small blood samples obtained by heel prick. The leukocytes were challenged by addition of carbon particles. A method of scoring is proposed, that of "effective phagocytosis," where the number of phagocytes containing 10 or more particles are distinguished from the total number of phagocytes. By this criterion, significant statistical differences exist between small premature infants and adult controls. In general, effective phagocytosis is directly proportional to birth weight. No confidence can be expressed concerning differences in phagocytosis between full-term newborn infants and adults. Considerations of phagocytosis scoring only mature polymorphonuclear leukocytes may be misleading, since myelocytic cells and monocytes are actively phagocytic and may comprise up to 20% of the total white blood cell count. Addition of normal adult serum to leukocyte-carbon suspensions resulted in a significant increase in effective phagocytosis in the premature infants. The phagocytosis-promoting factors in serum were identified in the α- and β-globulin fractions.

Discussion

MARVIN CORNBLATH, Baltimore: I should like to ask the author whether he follows the process of phagocytosis during the incubation period in any of his tests. I was wondering if serum factors had any effect on the motility of these particles.

LOUIS GLUCK: One of the disadvantages in using bacteria is that phagocytosis is slowed up by specific antibody. One of our reasons for choosing carbon as the challenging substance was one paper that showed that carbon is phagocytized four times as rapidly as bacteria. The use of carbon thus eliminated another important variable—antibody effect. If phagocytosis were vigorous it occurred almost immediately and proceeded to larger and larger amounts of carbon ingested rapidly. Our observations seemed, thus, independent of any antibody action.

Retrograde Aortography Under Hypothermia in Infancy and Early Childhood. HOWARD A. JOOS and JOHN L. JOHNSON,* Southern California.

In the diagnostic investigation of selected patients with congenital heart disease, retrograde aortograms were made under hypothermia in 27 patients between the ages of 2 months and 5 years. The method described demonstrates improved quality of results, increased safety to patients, and specific advantage over alternative diagnostic methods under certain conditions.

Body temperature was lowered in an ice bath or by application of plastic bags filled with ice cubes after induction of general anesthesia with nitrous oxide-trichloroethylene and succinylcholine.

* By invitation.

Range of body temperature during aortography was 26.5 to 35.5 C (79.7 to 95.9 F), average 33.1 C (91.6 F). An artery, usually the right brachial, was intubated with the largest metal cannula accommodated. Lead I of the electrocardiogram was monitored continuously during the procedure. Fifty per cent neutral diatrizoate sodium (Hypaque Sodium), about 1 cc. per kilogram of body weight, was injected rapidly under manual pressure, and simultaneous biplane roentgenograms were made, with use of an Elema biplane roll film changer at a speed of twelve 10×14 in. pictures per second for at least one second.

Retrograde aortography has been particularly helpful in the frequently encountered and difficult differentiation of patent ductus arteriosus or aortico pulmonary septal defect from ventricular septal defect in infancy. In addition, the method demonstrates coarctation of the aorta exceptionally well, whether or not congestive heart failure is present. The method is briefer than cardiac catheterization and superior to it in identifying patent ductus or coarctation in infancy, particularly with cardiac failure.

Hypothermia slows both heart rate and blood flow, improving visualization of contrast medium, both in intensity of contrast and in duration. As a result the accuracy and reliability of retrograde aortography are greatly enhanced. In addition, hypothermia has reduced the number of untoward reactions otherwise associated with the procedure, even in the case of young and seriously ill infants.

SAUL J. ROBINSON, San Francisco: We have lost some of our enthusiasm for retrograde aortography because open heart surgery is now available, and it is obvious that aortography for patent ductus only gives part of the answer. After the ductus is ligated or dissected there is often another lesion which still must be diagnosed by other procedures, such as cardiac catheterization. The time and effort involved in anesthesia and hypothermia for a diagnostic procedure such as aortography seem to me to be almost equal to that for cardiac surgery. I repeat that our enthusiasm for retrograde aortography, despite the fact that it has a very low mortality, is now being replaced in many cases by advocation of cardiac catheterization, except in those instances where the infant is very ill and the cardiac catheterization schedule is very full and one wants to do something for the infant if he has a curable lesion, such as a patent ductus, even though another lesion may be present.

PAUL R. LURIE, Indianapolis: Following the lead of Sones, of Cleveland Clinic, Dr. John Campbell and I at Indiana have studied extensively a combined technique of cardiac catheterization by the venous route and selective angiocardiography, using an image amplifier with moving pictures at 30 frames per second. This, we feel, permits the measurement of intracardiac pressures and oxygen contents, which can not be done, of course, with aortography. And at the same procedure and with the same mild sedation, which is, incidentally, meperidine (Demerol) and secobarbital (Seconal), it is possible to get angiocardiograms giving every bit as much data regarding the aorta as does retrograde aortography. This is done by injection into the left atrium, the pulmonary artery, or the aorta itself via a ventricular septal defect. Thus we can see the aorta and coarctations, both suspected and, even more important, unsuspected, as well as filling the patent ductus, often from both ends. With 30 frames per second speed, motion of the patient is no problem, nor is tachycardia. We certainly admire the superior photography obtained by Dr. Joos and Dr. Johnson with an Elema device. However, we have many reasons for preferring image-amplification movie technique. One is the ease of handling and study of these films as well as of filing, when one is dealing with just a small strip of film as opposed to many large pieces of cut film. Even more important is the perception of small jets and faint clouds of contrast medium, which is greatly facilitated by movie projection in the same visual field in rapid sequence. The small size of the field of view and the relative lack of definition as compared with Dr. Joos' method of photography are the present principal objections to image-amplifier methods. There are rapid developments in movie technique which we feel will soon surmount these objections, and we feel strongly that in the near future a combined technique of this sort which yields physiological and anatomical information at the same time will become the cornerstone of diagnosis in congenital heart disease.

GEORGE S. HUSSON, Syracuse, N. Y.: I would echo Dr. Robinson's comments, and in addition, with this beautiful equipment that Dr. Joos and Dr. Robinson have, giving them 12 pictures per second, I wonder if we are justified in adding another potentially hazardous procedure such as hypothermia to aortography, which in itself can be a hazardous procedure in the young infant, especially in congestive heart failure.

SAMUEL KAPLAN, Cincinnati: About four years ago we described our experiences with the use of hypothermia as an aid in the diagnosis of various types of congenital heart disease. This experience included some 20 patients. Since that time we have lost our enthusiasm for the use of hypothermia during aortography because we are able to get the required information by performing aortography under barbiturate sedation. One would agree with the previous discussions that in older children in whom intracardiac surgery is being considered the diagnostic procedure of choice is cardiac catheterization with selective cardioangiography.

HOWARD A. JOOS, Los Angeles: The varied opinions expressed emphasize that each person will choose investigative procedures according to his estimate of the diagnostic objectives, conditioned by his experience with the available alternatives and and his confidence in them.

I can add little to the conclusions of the presentation. Modest hypothermia does not appear to aggravate the risk of aortography. On the contrary, the procedure seems smoother and safer under these conditions. As a result, we are now reluctant to accumulate a control experience without hypothermia to prove the advantage of lowering body temperature. The total time required for anesthesia and for completion of aortography compares with that necessary for angiocardiography, and it is considerably shorter than that required for cardiac catheterization in our laboratory. In addition, hypothermia appears to improve the quality and reliability of retrograde aortography rather distinctively.

In the absence of readily available open heart surgery, our primary objective in studying acyanotic patients with flooded lungs is the reliable differentiation of patent ductus arteriosus. Aortography accomplishes this objective particularly effectively. A coincident intracardiac left-to-right shunt will not be identified, but it will not usually contraindicate closure of a ductus shunting in the same direction.

We have no experience comparable to that of Dr. Lurie with differential cineangiocardiography. Visualization of left heart and aortic structures, however, would appear inescapably affected by dilution through the pulmonary vascular bed when injection is made into the pulmonary artery. When the pulmonary blood flow is augmented by a left-to-right shunt, we might expect the effect of dilution to be exaggerated accordingly.

A Comparative Study of the Glycolytic and Pentose Phosphate Pathways in Red Blood Cells of Newborns, Prematures, Infants, and Children. RUTH T. GROSS and RUTH E. HURWITZ,* Stanford.

It is the purpose of this study to investigate the state of the metabolic pathways in the red blood cells at birth and to follow the transition to the adult pattern as reflected by measurements of certain enzymes.

Aldolase is an enzyme which is active in red cells and occurs only in the glycolytic pathway. We have found, in the full-term infant, that aldolase activity is high at birth and gradually reaches adult levels during the first 36 weeks of life. The premature infant has higher levels than the full-term infant.

Two other enzymes, active in red cells and occurring only in an alternate pathway of carbohydrate metabolism, have also been measured in infants and adults. These are glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase, which are involved only in the first two (oxidative) steps of the pentose phosphate shunt pathway. The levels of activity of these enzymes were also found to be high at birth, again taking approximately 35 weeks to reach the lower adult levels. The level in the premature infant is significantly higher than in the full-term infant. A few unexplained low values for glucose-6-phosphate dehydrogenase have been found in Negro infants. The highest values for all three enzymes were found in children with various types of anemias.

Discussion

SOLOMAN A. KAPLAN, Brooklyn: This is a very elegant demonstration of the possible existence of the hexosephosphate shunt in the newborn infant. I have a few questions to ask.

The first is whether there is any evidence that the other enzymes of this shunt are present in the newborn infant. As we know, for any metabolic pathway to be operative, all the enzymes have to be present. We know that in galactosemia only one specific enzyme is absent, and so persons with galactosemia are unable to handle galactose. Is there any evidence that, for example, transaldolase is present in the red cells of the newborn infant?

The second question I should like to ask is whether there is any correlation between the number of nucleated red cells and the measured activity of these enzymes. We know that the oxidative Krebs cycle is present in the red cells of those species which do have nucleated red cells; the human, having no, or very few, nucleated red cells, can not take the breakdown products of glucose further than lactic acid.

The third point I would like to make is rather a comment than a question, and that is the comment that aldolase—I am not speaking of transaldolase—aldolase really has some role, as far as I understand it, in the hexose monophosphate shunt. It is responsible for the equilibrium between sedoheptulose 1,7-diphosphate and tetrose phosphate and phosphoglyceraldehyde. I think the statement that aldolase operates only in the hexose monophosphate shunt might have to be modified in view of this evidence. Perhaps you would like to say something about that.

MARVIN CORNBLATH, Baltimore: I was wondering whether the standard of reference for activity was hemoglobin or the number of red cells. If the latter was used, were nucleated red cells counted?

A second question. What was the mode of delivery of these infants? We have found something

* By invitation.

very interesting in following sugars of newborn infants after administration of glucagon, a hormone which activates phosphorylase. Incidentally, we have been able to demonstrate phosphorylase activity in both newborn and adult red blood cells. We have found that infants delivered by elective cesarean section do not show a hyperglycemic response to glucagon, whereas all infants, both full-term and premature, delivered vaginally do. I was wondering whether the mode of delivery might have some effect on the activity of these other enzymes in the red cells?

VINCENT KELLEY, Salt Lake City: I have two questions I should like to ask. One of these is suggested, Dr. Gross, by one of your slides which showed levels of fetal hemoglobin and levels of pyruvic acid. However, I did not hear you mention the correlations that might exist between these two variables and would be interested in hearing more about this. Also, I wonder whether from your previous studies of the citric acid cycle and your present study you would care to say anything about the relationship that you might expect to exist between citric acid cycle activity and the activity of the enzyme systems of which you are speaking here.

DENMAN HAMMOND, San Francisco: I was particularly interested in this because of a similar interest in the hypoglycemic periods of premature infants. We had the opportunity to study a series of premature infants by means of epinephrine and glucagon tolerance tests done serially during the first few weeks of life, three to five times on each infant. We found that the response to both epinephrine and glucagon by premature infants of all weights was good at all ages, and yet there seemed to be a tendency to maturation of a somewhat sluggish initial response to these glycolytic agents. By the end of the first week the infants were having a much more adult type of response. They had a good hyperglycemic response even during their periods of rather marked fasting hypoglycemia. They also had a response from their fasting hypoglycemia before their diet had been sufficient to explain it on the basis of increased caloric intake.

I wanted to ask Dr. Gross if she felt that any of her studies might explain some of these differences, and whether she thinks this might be related to qualitative differences in glycolysis at this age.

I want to further ask whether she has had the opportunity to study serum values of aldolase and whether she can relate serum aldolase to states of hemolysis, such as erythroblastosis or other hemolytic anemias.

THOMAS H. SHEPARD, Seattle: I should like to ask if the carbonic anhydrase in the red cells was measured at the same time. I notice from our work and that of others that the blood carbonic anhydrase rises at about the same time that these enzymes drop.

DAVID YI-YUNG HSIA, Chicago: I should like to reemphasize the importance of genetic influences upon enzyme systems. In this paper, Dr. Gross has shown that the glucose-6-PO_4 dehydrogenase system varies not only on the basis of maturity but also with the familial background. The same variations undoubtedly are present in all enzyme systems. By bearing the genetic factor in mind, the variations can be more meaningfully segregated.

ROBERT SLATER, Toronto: Since Dr. Gross has been working in the anaerobic part of the cycle breakdown of sugar, I wonder what information there is about the effect of anoxia on this enzyme system?

RUTH T. GROSS, San Francisco: Dr. Kaplan, the only other enzyme assay we have done is the 6-phosphogluconic dehydrogenase. We have not attempted transaldolase or any of the other enzymes which are involved in the hexose monophosphate shunt. I feel we need to do more work to clearly delineate the fact that we are measuring shunt activity. I hope to start this very soon. My plan is to use differentially labeled glucose and to incubate it with the red cells and then try to decide from the pattern of CO_2 labeling whether or not we are getting shunt activity. I know that more work has to be done and that in a sense I am taking a liberty in talking about shunt activity when I have only given you one enzyme determination.

You asked about the number of nucleated red cells in the presence of these enzymes. It seems to me that every time I have the honor of getting up before these combined societies I somehow encroach upon a field about which I know nothing, namely, hematology—and it has happened again. I really am interested in carbohydrate metabolism, and somehow I get involved with red cells. I am not a good enough hematologist to do a decent blood cell count, and I therefore have not included the number of nucleated red cells or the reticulocyte count, and I wish I had. I wish someone who was interested would come and work with me and do this part of the study.

I was asked . . . I guess I was not asked, I was told that aldolase probably is not involved in only the glycolytic pathway. Perhaps this is so, but I think that its primary effect is in the glycolytic pathway.

Dr. Cornblath asked a very probing question, namely, what is our standard of reference for activity. This is an extremely difficult thing. We found ourselves working in a field in which not very many people had gotten into print yet, and so we more or less had our own choice as to what standard of activity to use. We used a standard which I no longer am satisfied with, and I would like to change it publicly now so that if anybody

else gets involved in this field we can be talking about the same thing. We have used 1 ml. of packed red cells and have referred everything to that. I think this is not very good and that we should add to that the hemoglobin content of that 1 ml. of packed red cells and refer to that as well as to the volume. Then I think we would have a little better standardization of activity.

I do not know about the mode of delivery of these infants, and I think it is an extremely worthwhile question, and one which I hope to pursue when I return to San Francisco.

I appreciate Dr. Kelley's questions because they give me the opportunity to say a couple of the things that time did not permit during my presentation. It occurred to us that possibly the level of enzymes would fall at about the same time that fetal hemoglobin dropped to normal values and that if this were the case it would make the study much less interesting. Therefore we did about 30 determinations of fetal hemoglobin along with our enzyme determinations and found that there was no such correlation. This was particularly striking in premature infants, where we had negative correlations in two ways: On the one hand, we had premature infants who still had high levels of fetal hemoglobin and whose enzyme levels were fairly normal; on the other hand, we had infants who still had high enzyme levels whose fetal hemoglobin had dropped. So apparently there is no clear-cut correlation with fetal hemoglobin.

As far as pyruvic acid is concerned, the reason I measured it was to see what correlation there might be with over-all carbohydrate metabolism.

I have been asked by several of the discussers what relationship I feel these results have with the over-all picture of carbohydrate metabolism in the newborn infant and what connection these observations have with previous studies that I have done in anaerobic metabolism. My feeling is that in this study I have probably been dealing with metabolism in the red cell alone and that probably this is not representative, unless it is purely fortuitous, of what is going on in the liver.

I have been asked how this fits in with citric acid cycle activity, which has interested me in the past, and I have been asked by Dr. Hammond about the relationship to his very elegant studies on epinephrine and glucagon in premature infants. Along with him, I really want to know what happens in the livers of these babies. I only started out using an accessible tissue, the red cell, but having done so I am fairly well convinced from our studies that we do not know anything yet about what is going on in the baby's liver just because we have some interesting observations in the red cells. I think this is strictly red cell metabolism.

We have not determined serum aldolase, only red cells, and in the studies that we have done in persons who have hemolytic disease, which I showed you in the last slide, all of our enzyme levels were high in every type of anemia which we tested.

We have not studied carbonic anhydrase; we do not know the effect of anoxia. I think they are very good questions.

Dr. Hsia, thank you for your comment about genetics. I think the babies with the very low enzyme levels probably have some genetic defect. Perhaps they are primaquine- or naphthaline-sensitive.

Thallotoxicosis: Neurologic Manifestations and Methods of Treatment. P. H. CHAMBERLAIN,* W. B. STAVINOHA,* H. DAVIS,* T. KNIKER,* and T. C. PANOS, Texas.

Fourteen children with thallium intoxication have been studied during the past year. All became ill following ingestion of a newly marketed pesticide containing 3% thallium sulfate in a crushed vanilla wafer base. All patients developed alopecia. Outstanding among clinical manifestations was evidence of involvement of the central nervous system, which occurred in 10 patients. The remaining four apparently suffered only alopecia. The multiplicity of neurologic involvement is indicated by the following: Weakness occurred in 9 patients; lethargy, in 9; ataxia, in 9; tremor, in 9; irritability, in 7; headache, in 6; coma, in 6; hyperreflexia, in 6; convulsions, in 5; and respiratory paralysis, in 4. Gastrointestinal symptomatology was not conspicuous. Two patients died; one was left decerebrate.

Diagnosis depends on a high index of suspicion, especially in cases with bizarre neurological disturbances before alopecia appears. Identification of thallium in the urine represents the only accurate positive diagnostic measure. A new technique for this determination has been developed, based on extraction of HNO_3-digested urine with diphenylthiocarbazone (Dithizone) and subsequent estimation in a flame spectrophotometer fitted with a photomultiplier tube. By these methods, amounts as little as 0.25μg. can be detected.

Methods of therapy described in current textbooks of toxicology have been quite disappointing (sodium iodide-sodium thiosulfate, diuretics, potassium iodide, cystine, pilocarpine). Neither dimercaprol (BAL) nor edathamil calcium (Versenate) was found to be an effective therapeutic agent in the present series. Sodium lactate was used in two cases with suggestive but inconclusive benefit. On the basis of Lund's (*Acta pharmacol. et toxicol.* 12:360, 1956) encouraging report of the protective and therapeutic effectiveness of Dithizone in experimental thallium poisoning (rats), this substance was administered with glucose to six patients, in dosage of 10 mg. per kilogram twice daily by mouth. Since very large

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doses are potentially diabetogenic or goitrogenic and may actually potentiate the toxicity of thallium, caution in dosage must be exercised. Clinical improvement was apparent in five of the six patients, although this improvement was not accompanied by increased urinary execretion of thallium. No toxic effects of any kind were observed. It is concluded that the results warrant further cautious trial of Dithizone in thallium intoxication.

Discussion

EDWARD L. PRATT, Dallas, Texas: In these societies it is customary to refrain from commercial names and advertising, but I wonder if it would not be helpful to all of us if the author would repeat again the name of this preparation and what it says on the label about it.

JAMES B. SIDBURY JR., Baltimore: Observations of response to therapy in two cases, one severe mercury poisoning and the other severe thallium poisoning, suggest a possible approach to such intoxication. In the first case there was steady deterioration during two weeks of corticotropin (ACTH) administration and marked objective improvement within 48 hours of the institution of therapy with the disodium calcium salt of edathamil. In the second case the edathamil effected no improvement but there was distinct objective improvement within 24 hours after intravenous hydrocortisone therapy. The combination of agents may prove to be clinically useful.

Mouse protection tests with the edathamil salt showed no effect for mercury or thallium poisoning, but studies using both corticosteroids and edathamil have not yet been undertaken.

I believe that Dithizone would be contraindicated in those Negroes with the genetic defect as described by Dr. Zinkham.

CHARLES U. LOWE, Buffalo: How soon after ingestion did the patient manifest signs of intoxication? Can the authors give us any idea of how much thallium must be ingested before signs of intoxication appear?

ROBERT KLEIN, Pittsburgh: Maybe I was wrong in interpreting the slides, but it seemed to me that when Dithizone was added not only did the secretion of thallium not increase but it seemed to decrease. Now, this may be a misinterpretation.

The question, then, is this: Does this possibly interfere with the determination of the material in the urine?

P. H. CHAMBERLAIN, Galveston, Texas: In answer to Dr. Pratt's question, I have a slide I would like to show.

We do not like to advertise Mr. Echols, especially in Galveston. This preparation, Echols' Roach Powder, or Kingtex, is very tasty; it smells very good, and it is just the right thing for toddlers to pick up. It is made in Corpus Christi and it sells for 60c an ounce. On the bottle it says roach, large red ant, small ants, and mouse poison.

Dr. Sidbury, we did not use cortisone on any of our patients. We found several references to its ineffectiveness in the literature. Concerning the use of Dithizone for Negro patients, it was used for two Negro patients in our series and we found no evidence of any hemolytic anemia during treatment or months after treatment.

Dr. Lowe, you asked when intoxication appeared. The earliest time that intoxication appeared in our patients was about 12 to 14 hours after ingestion. That was one of the cases in which we knew exactly when the ingestion occurred. In most of our cases the time of ingestion was surmised or calculated.

As to the question of how much thallium is required to produce this intoxication, I can say that nobody knows for sure. However, the dosage for epilation that was used in the past in the treatment of ringworm of the scalp in children was 8 mg. per kilogram, and with this dose many cases of intoxication occurred. Several workers have reported that dosage of half that amount, 4 mg. per kilogram, has resulted in intoxication.

Dr. Klein, I believe that the reason these slides tended to show that the thallium decreased after the onset of dithizone therapy was that most of these patients had been ill for one or two weeks before we saw them, and I think that the thallium excretion was decreasing anyhow.

The Obstructive Factors in the Pulmonary Hyaline Membrane Syndrome of Newborn Infants. JOHN M. CRAIG, KEVIN FENTON,* and DAVID GITLIN, Harvard.

Lungs from newborn infants dying with pulmonary hyaline membrane formation were compressed and then expanded in a bell jar under positive and negative pressures. During the period of expansion, latex, gelatin and India ink, plasma and India ink, and plasminogen-streptokinase and plasmin solutions were allowed to fill the trachea, bronchi, and alveolar spaces in the lungs. The latex-injected lungs were then subjected to corrosion and studied directly or imbedded in celloidin and sectioned at 100μ to 200μ. The gelatin-India ink and albumin-India ink and plasmin injected specimens were fixed in cold formalin, imbedded in paraffin, and sectioned. In selected areas reconstruction of serial sections was performed on the gelatin-India ink preparations. All of the injection studies and reconstructions show that the hyaline membrane is truly obstructive, preventing the filling of the alveoli, though the areas up to the membranes obstruction fill with ease. They show that, even though the alveolar ducts were greatly overdistended, no expansion of the terminal

* By invitation.

alveoli occurs, that little of the injection material enters the terminal alveoli, and that fine extensions of membranes can be traced into the terminal alveoli. These results confirm the early findings of Farber and Wilson (*Arch. Path.* 14:437; 450, 1932). In anatomically normal lungs, filling of the distended terminal alveoli can be accomplished only after repeated expansion and compression of the lung for removal of entrapped air bubbles, while in lungs with the hyaline membrane syndrome there is immediate complete filling of the expansible alveolar spaces. While activated plasminogen or plasmin will partially remove hyaline membranes from fresh-frozen dried and mounted sections of lung, distention of such fresh lungs with plasmin in saline or buffer at a limit of up to 380 units per cubic centimeter will not significantly remove the membranes after 1- to 12-hour incubation periods, though such instillations at levels of 600 units per cubic centimeter will cause definite alteration in the membranes at one hour and their disintegration at three hours of incubation.

Discussion

WILLIAM A. SILVERMAN, New York: Since in many examples of hyaline membrane syndrome the hyaline material is not uniformly distributed throughout the lung, and since the degree of atelectasis is often as severe in the areas where no hyaline membrane is seen as in areas where the material is abundant, I should like to ask Dr. Craig whether there was filling of the atelectatic portions of the lung where no hyaline membrane was present.

FREDERICK C. MOLL, Seattle: I should like to ask Dr. Craig about the utilization of the plasmin which was injected. Since there is a large amount of naturally occurring plasmin inhibitor in tissue, I wonder if the plasmin was inactivated in the lung or utilized in proteolysis although there was no change in the hyaline membrane. Further, was the presence of an inhibitor looked for in the control preparations?

STUART S. STEVENSON, Pittsburgh: We attempted to test the ability of bovine fibrinolysin (Parke, Davis) to prevent the formation of hyaline membrane in a small series of 15 guinea pigs which were subjected to 98% oxygen and intratracheal amniotic fluid. Previously, we had been able to make membranes in about 98% of guinea pigs under these conditions. The guinea pigs breathed a fibrinolysin aerosol constantly until they died, or up to a four-day period. Of the 10 guinea pigs, 13 developed thick hyaline membranes and the fibrinolysin seemed to be ineffective in preventing the formation of the membranes or in lysing them.

ROBERT E. COOKE, Baltimore: We have for the last two years tried plasmin in some clinical cases. This material was prepared according to the method of Kline and is a purified material which can be given intravenously. We have used it by aerosol. I can not give any evidence whatsoever to indicate its therapeutic effectiveness. I can only state that there was no obvious toxicity, at least in infants or somewhat older children. The dosage that we used was somewhat low, I think, according to this report, 100 units per milliliter was the material that was given by aerosol by intermittent positive pressure.

JOHN M. CRAIG, Boston: In answer to Dr. Silverman's observation, regarding the areas where the membranes do not occur, one thing that these reconstructions do bring out is that the areas without membranes are peripheral to the areas with membranes and yet these areas do not expand, because of the proximal obstruction. We had a similar uniform appearance throughout all the four or five specimens in which we got good injections.

In answer to Dr. Moll, we had difficulty with what we believed were inhibitor substances. We originally used plasminogen and added streptokinase and were unable to get any demonstrable effect. We have no evidence or data on the presence of inhibitor, although the very high dosages of plasmin which we had to use to remove the membrane certainly suggest that an inhibitor may be present.

Polarographic Studies in Premature Infants. MARTHA HALEY,* WILLIAM T. NEWSOM,* and HAL C. BECKER,* New Orleans. Introduced by R. V. PLATOU.

The advantages of using polarography as a means of determining the physiology of tissue oxygen are numerous. In the past, open-tip plane electrodes have been used only to obtain an estimate of relative "oxygen tension" changes. The need for some method of measuring the availability of oxygen to the cells of a particular tissue in a quantitative and continuous manner seems to be of primary importance. By manipulation of certain equations of physical chemistry and electronics, it became evident that it was feasible to measure the number of moles of oxygen available at a specific area in a variety of media.

By using specific equipment, a numerical factor can be determined which, when multiplied by the meter readings of that equipment, yields a number which we believe to be a close approximation of the number of moles of oxygen diffusing to the electrode area per second. This we have called oxygen availability (OA), and this has been shown to vary only with the oxygen concentration in a specific tissue *or* with differing diffusion coefficients of various tissues in specific oxygen concentrations.

* By invitation.

We have studied oxygen availability in the skins of 25 premature infants having birth weights from 470 to 2000 gm. Concomitant serial determinations of body temperature, systolic blood pressure, pulse rate, and respiration were also made at intervals during the first 72 hours of life. Differences between those infants who died and the surviving infants were difficult to interpret because of differences in care, particularly in regard to supplementary oxygen. Skin oxygen availability values in the normal premature babies without supplementary oxygen showed wide variation from baby to baby, which represented, in part, varying diffusion coefficients of the skin. For this reason, consistent relationship between skin oxygen availabilities and such determinations as blood pressure, pulse rate, respiratory rate, and temperature could not be shown. Several of the babies studied by specific experimental design exhibited to us interesting data regarding the physiology of skin oxygen. From observations made to this time on many babies, both normal and abnormal, we feel there is evidence that significant amounts of oxygen are absorbed through the skin. Our data indicate that oxygen from this source is not made available, at least to the skin of other parts of the body. In determining oxygen availability in the skin and by eliminating that amount of this value due to absorption through the skin, a useful index of cardiopulmonary function can be serially determined by varying the oxygen concentration of inspired air.

Discussion

RICHARD L. DAY, Brooklyn: As I understand this electrode, it works in this way: One impresses a fixed voltage on it, and a current flows only when there are oxygen molecules available for electrolysis, and flows in proportion to the number of molecules at the tip. The moment the current begins to flow the number of molecules adjacent to the electrode declines and a concentration gradient is set up. At first the current declines rapidly, and then it reaches a steady value, the level of which is determined by the diffusion characteristics of the tissue and the tension of oxygen.

Tension of a gas is related to the number of molecules per cubic centimeter. However, in the body, the situation is made complex by the presence of hemoglobin, which accepts a lot of oxygen at low tension and in a nonlinear manner. Serum, in contrast to hemoglobin, can only accept small amounts of oxygen, even at very high pressure. Under the conditions of the experiment, as I understand it, oxygen passes through tissue to the electrode tip from a source which could be hemoglobin or could also be serum, if the patient is breathing 100% oxygen. In rats I found that the galvanometer showed two and one-half times as much current flow when 100% oxygen was breathed instead of air. Only some 10% more molecules of oxygen could have been available, however. Perhaps I would understand the term "oxygen availability" better if Dr. Haley would say what the increase in galvanometer reading is when 100% oxygen is breathed and what the change in oxygen availability would be, by her definition, if the current should increase two and one-half times.

SAMUEL KAPLAN, Cincinnati: We, too, have been interested in the polarographic measurement of tissue oxygen availability and the mechanism of action of implanted solid cathodes. Some of the factors which affect current flow include cathode movement and local variations in tissue temperature and pH. Could Dr. Haley tell us whether she measured skin temperatures? These findings would not be as significant if the skin temperature changed during the measurements of skin oxygen availability.

It would also be of interest to know how long the cathode was implanted before readings were taken. Dr. E. C. Matthews, of our group, has measured oxygen availability in infants by implanting the cathode subcutaneously, rather than intradermally, and he found that stable readings were obtained only after a period of about 8 to 12 hours.

The significance of small changes in current flow has been of some concern to us. Perhaps slight movement of the cathode could produce a "stirring" effect and result in an increase in current flow.

MARTHA HALEY, New Orleans: Dr. Day, I believe that I can answer your question concerning the amount of increase in oxygen availability when 100% oxygen is breathed, since this value would be dependent upon so many variables in any specific instance, some of these variables being the diffusion coefficients of the tissue involved and also the rate of blood flow to the tissue, along with the capacity of the lungs to oxygenate the blood. It is because of these numerous variables, that are unmeasurable as such, that we came to derive the concept of measuring "oxygen availability." By the mathematical derivations shown, oxygen availability expresses the number of moles of oxygen diffusing to the electrode surface per unit area per unit time and, thus, to the tissue involved. This obviates the necessity of knowing the exact diffusion coefficient of a particular tissue, or rate of blood flow, or percentage saturation of the blood. Thus we are measuring a value which is a composite of these variables, which individually are either unmeasurable or difficult to ascertain at the present time. I can not at this time answer your important question concerning hemoglobin saturation and oxygen availability, for the above reasons.

Dr. Kaplan, we made simultaneous measurements of rectal, environmental, and skin temperatures.

We are not able to show any consistent polarographic changes with changes in these. As you know, Brink and Davies in 1942 stated that with temperature changes in the range of 27 to 37 C the solubility of oxygen and the diffusion coefficients vary inversely, thus making temperature corrections negligible. All of our temperatures fell within these ranges.

Our cathode, we found, gave us acceptable readings after having been inserted for from 20 to 45 minutes. We obtained acceptable readings until the cathode slipped out of position or until the period of study was over. In instances where the positioning became unsatisfactory, reinsertion was carried out in the standard manner.

We realize that any motion of the baby can affect the readings of the polarography. We used only the measurements secured when the infants were quiet. The others could not be evaluated.

New Method for Detection of Poliomyelitis and Other Antiviral Antibodies in Human Sera. HORACE L. HODES, HELEN D. ZEPP,* WALTER L. HENLEY,* and RUTH BERGER,* Mt. Sinai Hospital, New York.

We have reported that if the lower edge of a strip of filter paper is placed in a suspension of a virus the virus rises upon the paper and becomes distributed in a regular, reproducible manner. The experiments to be described show clearly that the upward spread of virus is decreased when serum containing specific antibody is placed in a band across the filter paper. Serum without antibody does not exhibit this effect. The "blocking" action of specific antibody has been observed with polioviruses and with six other viruses. The "blocking" of polioviruses by human sera which contain neutralizing antibody is type-specific.

Methods.—Whatman filter paper No. 3 is cut into strips 12 by 1.75 cm. Each strip is marked off by light pencil lines into 1 cm. spaces (numbered 1 to 12) and suspended from a rubber stopper and autoclaved. Poliovirus cultivated in monkey kidney tissue is diluted to a concentration of 100 TCD_{50} per milliliter in 0.85% NaCl containing 10% bouillon broth. Thirty milliliter of the diluted virus is placed in a sterile bottle surrounded by ice. The serum to be tested (previously inactivated at 56 C) is then distributed evenly over Spaces 3 and 4 of the filter paper. The paper is placed in the bottle containing the virus with only the lower half of Space 1 below the surface of the virus suspension. After one hour the strips of paper are removed and each paper space is cut off and placed into a monkey kidney tissue-culture tube. Tissue-culture tubes are incubated and observed for virus cytopathogenic effect in the usual manner. Neutralizing antibody titers of the sera used in the paper tests were determined by standard tissue-culture methods.

Results.—Sixty-seven successive tests (134 paper strips) with 14 human sera have given virtually identical results. Virus was detected by tissue culture on every wet space of every paper strip on which serum containing no antibody had been placed. In contrast, no virus was found above Space 6 on any of the paper strips treated with serum which contained type-specific poliovirus antibody. No virus was detected above Space 4 in the vast majority of such strips. The "blocking" action is demonstrable with as little as 0.025 ml. of serum, a quantity readily obtainable by finger puncture.

For practical purposes, the placing of a single paper space into a single tissue-culture tube gives correct information regarding the presence or absence of poliovirus antibody. This is true of Space 7, for example, in each of the 67 successive tests performed. When a pool of Types 1, 2 and 3 polioviruses is tested against a serum, only that type of virus against which there is no specific antibody in the serum can be detected high on the paper.

Summary.—A new method for the detection of poliovirus antibody is described. With a quantity of serum obtainable by finger puncture and a single tissue-culture tube it is possible to determine whether an individual possesses antibodies against all three types of poliovirus.

It is clear that three injections of Salk vaccine do not immunize all children to poliomyelitis. Furthermore, a fall in antibody titer occurs in an appreciable percentage of vaccinated children with passage of time, so that booster injections are required. It is suggested that the method described here may be a valuable screening test for distinguishing immune from nonimmune persons in a polio vaccination program which aims at complete protection.

Discussion

ROBERT WARD, New York: I should like to take this opportunity of expressing my admiration for this brilliant and imaginative piece of work. It seems to have both practical as well as possibly fundamental importance.

I have a question for Dr. Hodes which relates to the possibility of differences in kind as well as in amount of antibody during the early stage of poliomyelitis in contrast to antibody in the convalescence stage. Dr. Brunner in our laboratory has been working with the Dulbecco plaque method. The results suggest that such qualitative differences might be observed. I am wondering if, with the paper strip method, Dr. Hodes has any indication that perhaps such qualitative differences occur.

CHARLES A. JANEWAY, Boston: I should like to congratulate Dr. Hodes too on a beautiful piece of work, which is another example of how virological methods are being simplified to a level where

* By invitation.

they can be used not just as research tools but as practical means of studying disease by many persons.

I think one suggestion that might be made is that the reverse technique of tagging antibody instead of trying to tag the virus might increase the ease with which this method could be carried out. Namely, if the Coons technique, which is being used a good deal now for the localization of virus in tissue-culture cells, could be applied to the presence of virus in the upper portion of the strip, the blocking action of immune serum perhaps could be demonstrated without having to go through reinoculation of tissue culture for detection of virus.

DAVID T. KARZON, Buffalo: Do you have any indication as yet of the quantitative relationships of this method and other standard neutralization techniques such as metabolic inhibition? In other words, what is the sensitivity of this method?

Second, could you give us some of your thoughts on the mechanism of the antibody block of virus migration?

WILLIAM A. REILLY, San Francisco: If Dr. Hodes is disappointed in the ability to successfully tag this virus with P_{32}, I should like to give him courage by saying that he has an illustrious company. It is a hard job, from what little I know of it, to tag a virus or bacteria or even a chemical compound. For the interest of those who would like to know something about it in general, let me state that when you put a radioisotope against anything there is a possibility of disintegration from the internal radiation. Also, when working with iodinated human serum albumin, rose bengal, etc., we find by audioradiograms that within a week some of these compounds lose some of their radioisotopes, which are then free and a source of error of measurement. If there is elemental phosphorus in the system (in monkey tissue culture of poliovirus), this also is another complication. This is a problem for radiochemists. I should like to offer encouragement and congratulation on this ingenious method. When the method of tagging the virus is finally worked out, it will probably be very helpful.

ROBERT KLEIN, Pittsburgh: What is meant by distinguishing immune from nonimmune persons by antibody level?

DR. MARK, Chicago: I wonder whether these tests have been tried using whole blood on the strip, as it would seem to simplify the method further.

HORACE L. HODES, New York: We have not actually studied early antibodies as compared with antibodies after infection. We have some indication that things change in persons who have been vaccinated, and I think that what Dr. Ward has in mind is that post-vaccination antibody is not the same as neutralizing antibody. I think that this is probably going to turn out to be correct. We have several hints as to why this may be so. I can not go into them all now, except to say that when one titers out neutralizing antibody and the antibody by the strip method they come out differently in both directions. The strip method is more sensitive in detecting a small amount, apparently, of antibody, whereas the neutralizing method titers out higher.

We have not actually tried Dr. Janeway's suggestion of tagging the antibody. But with help from his department through Dr. Gitlin we tried to put fluorescent anti-γ-globulin serum on the paper strips after they had been exposed to virus and after the serum had been put on them, with the idea of seeing whether there would be a difference in the fluorescent pattern.

I hate to mention just a couple of experiments, but I will say that with the serum Dr. Gitlin sent us we were able to show a difference. There was a fluorescent band concentrating around Spaces 4, 5, and 6 in the presence of serum containing antibody. This was not so with normal serum. We have tried to make this fluorescent anti-γ-globulin. We have not been very successful; hence I am speaking only of a few experiments. We certainly will pursue this procedure, perhaps with Dr. Janeway and Dr. Gitlin's help.

Dr. Karzon asked about quantitative aspects, and I think I have answered in part. We got on to this because mice immunized with "Z" virus did not seem to show any neutralizing antibody in their serum and yet we were able to demonstrate an antibody by the paper-strip method. Perhaps it is a more sensitive method.

As regards the mechanics of the test, I think there are a good many possible theories. From the little hint that we have from the serum of Dr. Gitlin, there probably occurs a union along the strip of paper in optimal proportions such as occurs in the methods demonstrated by Drs. Oudin and Ouchterlony and others. There may be union and a sticking of the complex on to the paper, preventing the passage of virus.

Dr. Reilly, I am encouraged to learn that other people have had trouble tagging viruses and other things. What you say occurs I think is a part of our problem. We can not get over 6μc. per milliliter in labeling without destroying the virus.

As to Dr. Klein's question about antibody levels, when I speak of immune and nonimmune serum as judged by neutralizing antibody, I mean that nonimmune serum contains no antibody in 1:4 dilution, and such serum always has turned out to be negative on our paper strips. On the other hand, serum with antibody in 1:8 dilution has given a sharp positive result, and the second pair of sera, labeled C and D on my slide, is an example of this.

Finally, the question about whole blood. We have tried it, and I wish it worked. So far we can

not get it to work. We put blood from a finger stick across the paper, and the trouble seems to be that after the blood dries the paper does not become wet beyond it.

Nutritional and Chemical Pitfalls in the Treatment of Phenylketonurics by Means of Low-Phenylalanine Diets. DAVID Y. Y. HSIA, RICHMOND S. PAINE,* and W. EUGENE KNOX,* Harvard.

Although several investigators have shown the feasibility of maintaining children with phenylketonuria on a synthetic diet low in phenylalanine content, the long-term chemical and nutritional effects of such a diet have not been recorded. The present paper will report our experiences with 22 phenylketonuric patients ranging from 6 months to 60 years who have been maintained on such a diet for one year or longer.

It was found that complete chemical control was difficult to achieve, and up to six months' time may be required before the abnormal metabolites can be eliminated in the older children and adults. Regular determinations of plasma phenylalanine and urine phenylpyruvate were made throughout the year's time. It was found that, although the latter rapidly disappeared from the urine, normal plasma phenylalanine levels were not obtained until considerably later. This indicates the importance of getting frequent plasma phenylalanine determinations, and one cannot be confident that the diet is achieving its chemical aim without such controls.

Secondary rises in plasma phenylalanine in previously well-controlled patients occurred as a result of (1) slight excess of phenylalanine in the diet and (2) inadequate intake of calories or proteins. These were corrected in four stages by modifying the diet and improving the palatability and variety of food. The clinical and metabolic observations made during these study periods will be discussed in detail.

Our studies indicate that long-term treatment of phenylketonuria by means of a synthetic diet low in phenylalanine content can be achieved. However, it requires the collaborative effort of a team trained in clinical medicine, biochemistry, and nutrition for its proper execution.

DR. HORNER, Denver: On the basis of the work of Armstrong and his group in Salt Lake City we have followed 12 patients for varying lengths of time on the dietary regimen which he so clearly outlined. In none of these patients did we have any difficulty in bringing serum phenylalanine down to normal levels within three weeks and maintaining it at a normal level following this dietary practice. The urines of these children all became negative within two weeks of dietary treatment. The discrepancy perhaps between some reports of these children might be based on the fact that the level was not down to the normal range and it was not maintained there for prolonged treatment. Our own experience with children now reveals that two children who were aged 4½ years when treatment was started, for whom an IQ was really not obtainable, as they did not speak, were not toilet-trained, and were completely unmanageable and on their way to institutions, are now actually in kindergarten and doing extremely well, and two newborn infants, who are now aged 2 years and 1 year, respectively, by all standards are developing perfectly normally. Perhaps our results are at variance with this group because none of our patients have been institutionalized and they have been under very close, practically day-to-day, supervision medically.

DR. BLANEY, Birmingham, England: We are interested in this problem. I have taken over the study of the cases that Dr. Bickel originally reported for the last four years. I am a little surprised at Dr. Hsia's difficulty in the initial control of these patients. Using a diet—not a synthetic diet but a diet with a phenylalanine casein—we have had no difficulty reducing phenylalanine to normal levels within two or three days, provided that the level has not exceeded a figure of about 15 mg. per kilogram per day. Very similar to the figure that Armstrong reported about two years ago, and rather unexpectedly, we found that the calory intakes in children exceeded a value of about 90 Cal. per kilogram per day. This, of course, is much more than one would expect. We found that the calory requirements were particularly susceptible to changes in reduction of carbohydrate; that is to say, reduction of carbohydrate below certain critical values produces high plasma phenylalanine levels; reduction below this level, isocaloric reduction, does not do the same thing.

I want to ask Dr. Hsia if he would perhaps tell us a little bit more. It was not quite clear from his dietary sheets whether these referred to calories and milligrams of phenylalanine per kilogram. If so, the amount of phenylalanine he was giving is about a third of what we give and about a tenth of what is usually estimated as a minimal requirement daily.

JOHN A. ANDERSON, Minneapolis: I am certain that we shall be indebted in the future to Dr. Hsia for this presentation on the detailed aspects of diets for children with phenylpyruvic oligophrenia and that we await with interest the publications of the details. It may be of interest to relate the case of a small infant in which dietary adjustment was somewhat difficult and led to secondary nutritional problems. We have recently studied an infant with phenylpyruvic oligophrenia in which the diagnosis was established at 5 weeks of age. The phenylalanine content of the diet of this infant was kept

* By invitation.

low, in accordance with the recommendation of previous reports. The protein intake was equivalent to 15% to 18% of the total calories. The infant did very well over the next few weeks, with essentially normal blood values, indicating satisfactory control. However, hypoproteinemia developed insidiously and edema became evident. It was found necessary to increase the protein intake of casein, which also, obviously, increased the phenylalanine. Thus it appeared that we had given inadequate dietary protein, necessary to meet all of the baby's requirements, in order to keep the phenylalanine intake minimal.

There was an incidental finding in this baby as well as in one other which may be mentioned here, as others may also have observed this. Somewhat coincident with the development of the hypoalbuminemia, a rather severe agranulocytosis was noted. This was not associated with infection. The granulocyte level returned to normal, however, sometime after the hypoalbuminemia was corrected. Incidentally, this baby, now over 1 year of age, failed to become the classical blond blue-eyed infant, as did an affected sibling now 3 years of age. The developmental capacity of the infant is perhaps around 80% to 90% of normal.

DENMAN HAMMOND, San Francisco: I was very interested to hear Dr. Hsia tell of the finding of anemia in his patients. Several months ago I was asked to see a phenylketonuric child who was managed by Dr. Langdell, of San Francisco, who may be here today. The child had a chronic anemia, with a hemoglobin level of about 7 or 8 gm. per 100 cc. He had a very hyperplastic bone marrow, with copius quantities of hemosiderin on Prussian blue stain. He had a rather elevated serum iron value and somewhat depressed iron-binding capacity of the serum; his hemoglobin was normal by electrophoretic analysis, and there was no elevation of fetal hemoglobin. The marrow was suggestively megaloblastic. This child was treated with folic acid and ascorbic acid and had no response at all after several weeks of therapy. Thereafter vitamin B_{12} was added, and he continued to show no response. During this period he was on a diet with a very low level of protein intake, and when no further changes in the regimen were instituted except for an increase in his protein intake he responded within the next two and a half weeks from a hemoglobin level of 7 gm. per 100 cc. to one of 11 gm. These latter values were not obtained in our laboratory, but we are tempted to speculate that the protein content of the diet might influence the ability to manufacture hemoglobin or that some undefined maturation factor usually associated with protein intake might be implicated. I wonder if Dr. Hsia had the opportunity to observe fluctuations in the level of anemia or other hematologic changes related to the changes he made in the diet.

DR. LANGDELL: I should like to comment a little further about the case Dr. Hammond has referred to.

In addition to the hematological problem we have also had a complication that has not been mentioned, and that is the phenylalanine level being extremely low in spite of our increasing the protein, the increase of protein which resulted in a response of reticulocytosis and increase in hemoglobin. The child still has less than 0.1 mg. of phenylalanine per 100 cc. in spite of everything, when we are giving him about 50% higher amounts of natural protein now than are recommended in most of the instructions. I would like to know if Dr. Hsia has had any complications in which the blood phenylalanine remained at much lower than normal level.

STEWART H. CLIFFORD, Brookline, Mass.: I should like to get on the record the very practical suggestion of Dr. Richard Maisland, of Bowman-Gray, that we should incorporate in every newborn examination a ferric chloride test on the urine.

NIELS L. LOW, Salt Lake City: Firstly, you raised the question about method. The method that Dr. Armstrong uses for phenylalanine determination is different from the one used in Boston. We use a modification of the Kapeller-Adler method in Salt Lake City.

I also want to agree with Dr. Horner that, at least in Salt Lake City, it usually does not take longer than two weeks to get a phenylalanine level to normal and to eliminate all of the normal metabolites from the urine, as determined by paper chromatography.

We have had trouble with anemia only when the blood level went too low. We have had some children in whom the level went below 2 mg. per 100 cc., and then we had difficulty.

The question about diagnosing phenylketonuria in the newborn period has some problems. We have had children in whom we were able to demonstrate in the urine that phenylketonuria was present as early as the age of 6 days. We also have had one child in whom it took 35 days before the first abnormal metabolite appeared in the urine.

DR. MCLOFF, Seattle: We have had about five children on the diet, and it has taken about two or three weeks to reduce their phenylalanine to normal. Excellent acceptance of this diet resulted when we began to use the Waring Blendor and used considerable amounts of banana extract to flavor it. One child, though, did develop a low serum protein, which was corrected by increasing the amount of the casein hydrolase in the diet.

RICHMOND S. PAINE, Boston: Perhaps I could be permitted to answer a couple of the questions which concern developments since Dr. Hsia moved to Chicago. We have had, as others mentioned, less difficulty in getting prompt control of plasma

phenylalanine levels in young infants treated at home as outpatients, but the institutional experience is, unfortunately, as stated.

The hemoglobin levels, determined at monthly intervals, were observed to fall equally in the control and in the experimental groups. This had no correlation with plasma phenylalanine levels, and the fall was unremitting to the vicinity of 7 to 10 gm. per 100 ml. of blood in the majority of children. The phenylalanine levels when extremely low (0.4 mg. per 100 cc. or less by our method) were followed by supplementation of the diet with 1 or 2 oz. of milk daily, but we had no clinical difficulties associated with the low levels noted.

I might mention that, in regard to diagnosis in newborns by plasma phenylalanine determinations, we have had the experience of following one heterozygous mother through a pregnancy and following her subsequent blood levels. Her clinically normal child's blood levels have now fallen to normal. Both mother and child had plasma phenylalanine levels to about 7.5 mg. per 100 cc. (in comparison with our top normal of 2 mg.) for 2 months after delivery. One might have mistaken this child for a phenylketonuric in that period.

JULIUS B. RICHMOND, Syracuse, N. Y.: I think we all appreciate the caution that Dr. Hsia exercised in commenting on changes in the intellectual level of these patients. I think it is also important to introduce the whole matter, which has been commented on only slightly, of age levels at which diagnosis was made and dietary therapy was instituted and the relationship of these factors to the social and emotional experiences of the person. I think that this does emphasize the point that Dr. Clifford just raised, and that is the importance of early diagnosis. From what we know about intellectual growth, it would seem extremely unlikely that patients who had been subjected to institutional environments for long periods of time in early life and were treated only at a later age would manifest much change in intellectual growth. This emphasizes the importance of early diagnosis and early therapy if one is to hope for improvement in the direction of positive intellectual growth.

HARRY A. WAISMAN, Madison, Wis.: I should like to be permitted to remark too on this problem, since it is of interest to me. The diet administered to these patients is, of course, a very poorly tasting one. In a modified synthetic diet such as Dr. Hsia has presented here today, taste is important; also, the administration of extra vitamins ought to be provided for carefully in order that the components of the diet will be sufficiently utilized.

Now, the dietary amino acids will vary a great deal, depending upon the source of the protein hydrolysate. Recently, in addition to the more commonly accepted casein hydrolysate, there is available a beef hydrolysate in which other amino acids are probably as well distributed, or maybe even better distributed, so that one does not fall into the trap of imbalance of amino acids when one is providing an already deficient diet. You will remember that these amino acids, these casein hydrolysates and beef hydrolysates, are made by absorption through charcoal. One must be careful in presuming that only phenylalanine is removed.

In utilizing phenylalanine antagonists in rats we have had much experience in the production of a pure phenylalanine deficiency. This not only requires a reduced protein intake but also requires the administration of β-2-thienylalanine, an antagonist for this particular amino acid. It is difficult to produce a complete deficiency of the plasma phenylalanine unless one uses a low-protein diet in addition to the phenylalanine antagonist, such as a 9% casein instead of 18% or 20%.

Now, if one wants to overcome this antagonism by the administration of phenylalanine, it is not a simple stoichiometric relationship; one can not give equimolecular amounts of phenylalanine to overcome the deficiency.

In all of the phenylalanine-deficient rats that we have used, we have never seen any anemia. This is of interest to me because, in contrast to some of the comments made today, the diet also—I must refer back to the diet—may include sources of protein which are not obvious. For example, potatoes contain a fair amount of protein and so do cereals. Now, all these little amounts add up sufficiently to make one very careful, when adding other food sources to an appropriately deficient diet.

DAVID YI-YUNG HSIA, Chicago: It should be emphasized that the purpose of the present presentation is to illustrate the practical difficulties of reducing phenylalanine levels in the plasma in an institutional environment. In our first slide, we showed that in the hospital we have very little difficulty in bringing plasma levels down to normal levels.

We do not question that there is a great deal of variation in the mental development of phenylketonuric children. We ourselves have encountered a patient with near-normal intelligence and know of several others. Also, institutionalization undoubtedly lowers the IQ, because of the lack of stimulus. However, it should be emphasized that, until the present study, all reported cases have not had adequate controls. It is unnecessary to point out the dangers of drawing overly-enthusiastic conclusions from uncontrolled data.

The total phenylalanine intake in Stage IV was based upon 400 mg. daily for a 150 lb. man. This is approximately half the daily requirement for a normal adult. Balance studies on minimal phenylalanine requirements in phenylketonuria will be published shortly.

With increasing evidence that the low-phenylalanine diet is useful only in very young infants, we must turn more and more to early diagnosis. Dr. Paine and I have recommended to the Mental Health Section of the American Academy of Pediatrics that the ferric chloride test should become a routine procedure at one month of age. Also, more effort should be made to detect heterozygous carries of phenylketonuria (*Nature*, London 178:1239, 1956).

In summary, I should like to say that we want to urge some caution about the use of the low-phenylalanine diet. We are not at this point ready for every practicing physician to administer the diet, and it should be done only under rigidly controlled laboratory conditions.

Sodium-Losing Material in the Urine of Newborn Infants. R. KLEIN, P. TAYLOR,* C. PAPADOTOS,* Z. LARON,* D. KEELE,* J. FORTUNATO,* and C. BYERS, Pittsburgh.

We reported one year ago to this society a single demonstration of a sodium-losing material in the urine of newborn infants. Since then we have demonstrated this material on six more extracts under varying conditions of assay. Amounts of material of the order of magnitude of 100μg. have produced the sodium loss, further strengthening the feeling that the material probably is a metabolite of the active hormone. At the present time efforts are being made to collect enough of the material and purify it so that it can be identified. The material has been characterized by its chromatographic behavior, staining and chemical characteristics, and ultraviolet absorption.

Discussion

WALLACE W. MCCRORY, Philadelphia: I would appreciate hearing from Dr. Klein a little bit more about the significance of the sodium excretion. I take it that these are not adrenalectomized rats. Do you have figures on changes in GFR that occur in the rats? Could changes in GFR be important in producing the sodium excretion?

CLAUDE J. MIGEON, Baltimore: If I understood it correctly, hydrolysis was the first step in this work, and, consequently, the compounds studied were free steroids. However, the substances actually excreted were conjugated, and so were the compounds in the circulation. Therefore, one would have to assume either that the conjugated compound has the same properties as its free form or that the free compound is present in the circulation as well as its conjugated form.

Secondly, the fact that corticotropin increased the excretion of this compound does not necessarily mean that it is a salt-losing steroid, but probably that corticotropin increased the production of this metabolite.

Finally, I understand that it is rather difficult to determine salt loss in rats, while, on the other hand, it is easy to quantitate salt retention, and so numerical values should be interpreted with care. Furthermore, salt loss in rats might not be necessarily related to salt loss in humans.

RICHARD T. SMITH, Minneapolis: I do not feel qualified to evaluate these data, but I should like to ask a question. How young were the newborn infants from whom the urine specimens were collected? If it was in the first 24 hours, I wonder if Dr. Klein has data on urine from the mother at or shortly after delivery.

R. KLEIN, Pittsburgh: Dr. McCrory, these were intact rats, as I mentioned. We have tried this in adrenalectomized rats. On two occasions, I think the results were comparable, but I hesitate, the variability of the excretion made it undesirable, to go further. Cortisone, of course, by increasing GFR will cause a Na diuresis in adrenalectomized animals.

The creatinine excretion, which is the closest I have to the measurement of glomerular filtration in these rats, did not change. These values were all reported as sodium per milligram of creatinine; they could have been reported as sodium milliequivalents per day or in relation to potassium; in other words, the same relationship holds. There is no significant change in potassium secretion, and I think this may answer the problem about filtration.

Dr. Migeon, we have run these same studies on urine that had not been hydrolyzed. In other words, we studied the steroid that was excreted in a free state and we got the same results. This is not of great significance, because a newborn infant does not conjugate well. I know that this is heresy and that the Salt Lake City group has argued the other way, but I think that our results are suggestive if not conclusive that the newborn infant does not conjugate well. Furthermore, Dr. Walters' and Dr. Zeulzer's groups are going to report later on in this meeting on other work that shows the same thing. I would assume that since the free form appears in the urine it also is present in the circulation. As I mentioned before, I have a hunch that we are dealing with the metabolite of the more active form, which one would expect to be free in the circulation in any event.

Secondly, in answer to the question about the significance of the increase of this material after corticotropin, I would have to agree that corticotropin could have increased the production of this metabolite of a normal hormone. However, I think that your objection is semantic. If the metabolite causes sodium diuresis, even though it is not secreted as such by the adrenal, then this

* By invitation.

metabolite is a salt-losing material. I would not press this point, since we have no information to prove whether this is a metabolite or a material that is secreted as such from the adrenal.

In answer to the third question, it is difficult to determine salt loss in rats, but the figures were statistically significant and the excretion of salt increased dramatically in the instances presented. The dotted lines represented two standard deviations on either side of the mean. Thus, in the cases presented, in all but one instance the sodium excretion was greater than the mean by three or four or more times the standard deviation and was in half the cases double the preceding and following days' excretion of sodium in the same rats. Salt loss in rats, as you say, might not necessarily be related to a similar loss in humans. The final answer to this question can only wait until enough material is available for purification, identification, and injection into humans.

Dr. Smith, the premature infants that we used, and we used premature infants merely because we were able to collect urine from them and they stayed in the nursery, were all a week or more of age. We have found that the corticotropin response in the sense of sodium diuresis does not disappear usually by a month, and we have some information on children 2 months of age who still respond this way. I do not know what the upper limit is.

Dr. Calcagno, I am not sure about your question. I have no information on solute excretion or possible loading effects. The K excretion did not increase, which might be against a solute diuresis. However, I have no evidence suggesting any relevance to osmotic loading.

Studies on the Mechanism of Ketosis in Infants and Children. MARGARET L. WILLIAMS,* ROBERT KAYE, and MITIO KUMAGAI,* Pennsylvania.

This report presents certain aspects of a study designed to further elucidate the mechanism of the unusual resistance to ketosis displayed by young infants. The data reported here deal with the relationship of age to the accumulation of ketone bodies on fasting and following fat infusion, with the possible role of age differences in renal excretion of ketone bodies as a contributing factor to the relative freedom of young infants from ketonuria, and, finally, with the rate of utilization of injected β-hydroxybutyric acid. The latter approach was planned in order to evaluate the possibility that the infant's resistance to ketosis might reflect a more rapid rate of utilization of ketone bodies as a manifestation of his over-all increased metabolic rate.

Blood levels of acetoacetic acid and β-hydroxybutyric acid were measured in 31 subjects fasted 24 hours and in 51 subjects given intravenous infusions of a standard load of fat. The children ranged in age from 10 days to 10 years and included 36 infants under 3 months of age. Under each of these conditions, ketone body accumulation was significantly lower in the subject under 3 months than in those from 3 months to 3 years. Beyond this age a progressive decline in ketone body response was seen. The resistance to ketosis in young infants is not complete, as shown by the development of high blood ketone levels in two infants of less than one month of age who received fat intravenously.

Simultaneous renal clearances of endogenous acetoacetic acid and β-hydroxybutyric acid and of endogenous creatinine were studied in eight subjects of various ages. Reabsorption of filtered ketones was found to be greater than 97% with blood levels of approximately 20 mg. per 100 cc. at all ages.

The rate of disappearance of acetoacetic and β-hydroxybutyric acids from the blood following a single intravenous injection of a standard quantity of Na β-hydroxybutyrate based on weight was studied in 12 infants and children. First-order velocity constants were calculated from serial determinations of blood ketones. The rate of disappearance did not appear to vary with age.

On the basis of present information, the resistance of young infants to ketosis and ketonuria does not appear to be based on differences in renal excretion or in rate of utilization of ketone bodies but rather to differences in rates of production.

Discussion

WALTER HEYMANN, Cleveland: I personally am grateful to Drs. Williams and Kaye for reviving a problem of important pediatric interest that has been lying idle for almost two decades. When we studied this problem, many years ago, no methods for blood ketone determination were as yet available. We arrived at the same conclusions that Dr. Williams and Dr. Kaye have arrived at.

As to the age disposition to ketosis, our studies led us to believe that the beginning of a disposition to ketosis was around 6 to 7 months. The peak in our studies was reached a little later, as Dr. Williams and Dr. Kaye indicated, namely, in the age group of 6 to 9 years. That very well may be connected with the different methods used in the two studies. That young infants in the age group as yet not predisposed to ketosis may develop good degrees of ketonuria was first pointed out by Schiff, who underlined the fact that dehydration helps to induce ketosis in infants otherwise not prone to develop it. I wonder whether the instances of marked ketosis that Dr. Williams and Dr. Kaye have seen in two young infants were in infants that were dehydated. The one that Dr. Williams mentioned suffered, I believe, from diarrhea.

* By invitation.

As to the problem of how to explain the noted age disposition to ketosis, Dr. Williams has outlined two possibilities which deserve very serious consideration. A third one is a possible greater stability of glycogen in liver or muscles of the very young infant when exposed to carbohydrate starvation. We tested that hypothesis in rats and in dogs and did not confirm it experimentally. The rate of glycogen disappearance in the liver under conditions of starvation was the same in the young group as in the older rats and dogs. We know, however, of many important differences in carbohydrate metabolism in young infants as compared with older children. To my knowledge, we do not know as yet of essential differences in fat metabolism in these age groups. Thus, I believe that further study of age-conditioned differences of carbohydrate metabolism rather than fat metabolism may contribute to the solution of this interesting problem.

SAMUEL BESSMAN, Baltimore: I should just like to point out that there is a very important difference between the metabolism of β-hydroxybutyric acid exogenously administered and that endogenously produced. For example, the brain has a tremendous fat oxidation but does not produce free acetoacetic acid or β-hydroxybutyric acid, because they can not be separated from coenzyme A. Since the brain has no deacylase for these CoA complexes, free β-hydroxybutyrate is oxidized without the intermediation of coenzyme A, whereas the endogenous acid is produced and oxidized mainly as the CoA-bound material.

SAMUEL SPECTOR, Cleveland: I should like to raise the question as to whether there was any determination of rate of utilization of the fat in the various age groups, because this may be an important factor in the development of ketosis. It has become apparent in the study of the endocrine control of fat metabolism in rats that hypophysectomy, which decreases the tendency to ketosis, causes an increase in utilization of fat. In converse, when cortisone is administered to normal rats and an accumulation of ketone bodies occurs there is a decrease in utilization of both endogenous and exogenous fat. In the one instance where the infant following recovery from diarrhea readily developed ketosis, the endocrine status of that child may have been a factor.

MARGARET L. WILLIAMS, Philadelphia: I appreciate the comments and interest in our paper. Actually, our first interest in this subject was stimulated by Dr. Heymann's early work on the problem.

We, too, have wondered what part a differing carbohydrate metabolism plays in the resistance to ketosis of the younger age group. The probable preponderance of an anaerobic type of metabolism in newborn infants might conceivably provide this group with an enhanced supply of Krebs cycle intermediates, owing to the greater production of pyruvate, but we of course have no evidence that this is the case.

Dr. Bessman, we did not study the pantothenic acid or total vitamin intakes in these children. We tried to choose well-nourished subjects for the experiments, and presumably most of them had received good vitamin intakes, but we have no actual data of this sort.

Dr. Spector's observations of decreased utilization of fat with cortisone are interesting and may have bearing on the wide individual differences in the development of ketosis by these convalescent patients of each age group. The 7-week-old infant who developed the spectacular ketosis seemed to be largely recovered from diarrhea—certainly as much so as many subjects who did not show such a reaction. This and other individual variations may reflect alterations in hormonal balance in the patients studied.

Anatomic and Physiologic Studies in Patients with Endocardial Fibroelastosis. LEONARD M. LINDE,* FORREST H. ADAMS, and BERNARD J. O'LOUGHLIN,* U. C. L. A.

Endocardial fibroelastosis as a cause of severe cardiac hypertrophy in infants is being recognized with increasing frequency. The diagnosis can be suspected clinically, but confirmation must be made at necropsy. We should like to report an angiocardiographic sign which is helpful in the antemortem diagnosis of this entity. In addition, the results and implications of in vitro studies of involved endocardium and myocardium will be discussed.

Previous angiocardiographic studies in fibroelastosis have emphasized the dilated left atrial and left ventricular chambers, the hypertrophied ventricular wall, and the delayed left heart emptying. We have measured systolic and diastolic left ventricular volume in four patients with this disease. Biplane angiocardiograms were taken at 6 to 12 frames per second after injection of 50% sodium diprotrizoate (Miokon sodium) into the main pulmonary artery.

These angiocardiographic studies revealed a complete loss of left ventricular contractility. In addition to delayed emptying, the dilated left ventricular chamber presented an unchanging contour during successive phases of the ensuing cardiac cycles.

In an attempt to understand the mechanism of this internal cardiac splinting, length tension studies were performed on the endocardium and myocardium obtained at postmortem examination of one of the patients dying of fibroelastosis. The

* By invitation.

greatly thickened endocardium produced marked limitation of myocardial contraction or stretching.

This inability of the left ventricle to contract adequately is undoubtedly just one pathophysiological aspect of this puzzling entity. Work in progress includes similar in vitro mechanical studies and exploration of coronary and thebesian circulation in endocardial fibroelastosis.

Discussion

JOHN J. OSBORN, San Francisco: Dr. Linde, those are beautiful angiocardiograms. You almost convinced me that the heart does not have to pump to keep the patient going, or that the left atrium perhaps can do it all alone, and yet as I think about it it seems to me that in theory the heart has got to contract to keep the blood going out the aorta. If we assume a certain output is necessary out through the aorta, then the linear contraction of the heart must be related to the heart rate and to the heart size, and I do not clearly understand why the heart with fibroelastosis looks different in the angiocardiogram than the very greatly dilated heart in failure caused by coarctation, for instance. It seems to me that the linear contraction in both cases must be the same if blood is still going out through the aorta at roughly the same rate.

ROBERT BRENT, Philadelphia: In the past three years at Walter Reed we have seen 31 cases resembling left aberrant coronary artery disease or endocardial fibroelastosis. In this group seven are alive. The 24 autopsies have indicated to us that differentiating these two entities is difficult. There are three cases of left aberrant coronary artery disease which were called fibroelastosis. These were cases in which the ECG findings of coronary artery disease were absent. I was wondering if you think that you can differentiate every case of endocardial fibroelastosis and left coronary artery disease and whether these two entities might give a similar angiocardiogram.

GEORGE S. HUSSON, Syracuse, N. Y.: I should like to ask the authors whether they have been able to correlate this sign with respect to the left ventricular splinting with the electrocardiographic findings as originally demonstrated by Lambert, of Buffalo, about three years ago, in which he demonstrated definite clinical and autopsy correlation, using the patterns over the left side of the heart.

LEONARD M. LINDE, Los Angeles: The first question concerns the specificity of the described angiocardiographic sign. Idiopathic myocarditis and cardiac glycogen storage disease might be differentiated on clinical grounds. We feel that an aberrant left coronary artery might be demonstrated by injection of the dye, under pressure, into the main pulmonary artery. We have not yet had an opportunity to study these other diseases angiocardiographically. One of the slides did demonstrate that this sign was not related to left ventricular failure or to left ventricular hypertrophy per se. Studies in aortic stenosis have revealed prolonged left ventricular dye retention but definite systolic and diastolic volume and contour change.

In answer to Dr. Osborn's question, there is no measurable volume change in systole and diastole. This coincides with other evidences of low cardiac output, poor aortic filling, and, frequently, congestive failure and death. Left ventricular output may be brought about by reduced but definite left ventricular contractions, possibly aided by left atrial forces. We do know that in these studies dye remained in the left ventricle for a definitely prolonged period.

Dr. Husson, in our experience, electrocardiographic differentiation of the various cases of left ventricular hypertrophy in infants has not been possible.

Deficiency of a Previously Unrecognized Serum Coagulation Factor Occurring in Five Siblings. ARTHUR E. MCELFRESH,* Temple. Introduced by JAMES B. AREY.

Several instances of faulty coagulation owing to a lack of factors present in serum have been described. There are now at least three such factors which are adsorbed by barium sulfate and which influence thromboplastin formation as measured by the thromboplastin-generation test; these are PTC, Stuart factor, and Factor X of Duckert. By cross correction studies another possible factor, serum Factor C, has been proposed by Grieg et al. The present report is concerned with five siblings, two males and three females, who have a mild hemorrhagic diathesis; a sixth sibling has a history of bleeding but has not been studied. The parents, who may be consanguineous, show no abnormality of clotting.

The deficiency of the factor is only evident in the thromboplastin-generation test. It is absent in all five siblings studied. The factor is present in normal serum, is absorbed by barium sulfate, is labile on heating to 60 C for three minutes, but is stable for two weeks at room temperature. It is present maximally in the 0 to 33% ammonium sulfate precipitate from serum and is not depressed by bishydroxycoumarin (Dicumarol). By cross-correction studies it has been shown not to be PTC, Stuart factor, PTA, or the Hageman factor. The stability at room temperature as well as the lack of effect upon its production by bishydroxycoumarin would militate against it being the factor described by Duckert. We believe that we are dealing with a deficiency of a serum coagulation factor which may be unique. However, the possibility that this factor may be the same as that described as Factor

C by Grieg et al., from South Africa, cannot be excluded. We have tentatively named this factor the Car factor after the family in which it has been found to be deficient.

Histories of the five affected members will be given, and the physical and biological properties of the deficient factor will be described.

Discussion

DR. VALLEE, Havana, Cuba: I should like to know whether Dr. McElfresh has investigated the condition of his factor, the Car factor, in the blood of newborn infants, because of the known deficiency of PTC existent in that.

DR. GIBBONS, Denver: I should like to congratulate Dr. McElfresh on what seems to be a very clear demonstration of this factor. I should like to ask whether he has tried putting the serum of either parent in the system with the patient in the hopes of perhaps demonstrating a defect that certainly should be present in one of them.

ARTHUR E. MCELFRESH, Narberth, Pa.: I should like to thank the discussers for being so kind. It took us a long time to gain courage enough to feel that this factor was unique.

To answer the question from Havana, no, we have not studied the newborn levels in comparison with these patients. We run into the same problem I am sure everyone else does; one can bleed these persons just so long. To get the original five we had to have a family reunion and serve spaghetti and wine and talk them into it, and they are getting a little tired of being stuck. We do have some lyophilized serum which we can make available for persons who feel they have a patient with a similar defect and would like to cross test with our patient.

In answer to Dr. Gibbons' very good question, we have tested the parents against only one of these patients. It was our feeling that the content in the parents was equal to that of normal serum.

Metabolism and Placental Transmission of Hydrocortisone (Cortisol) During Pregnancy, Near Term. CLAUDE J. MIGEON, JEAN BERTRAND,* PATRICIA E. WALL,* ROBERT S. STEMPFEL,* and HARRY PRYSTOWSKY,* Johns Hopkins.

The present work is an attempt to elucidate the metabolism of hydrocortisone during pregnancy, near term, and to study the placental passage of this hormone.

It was found that the intravenous administration of hydrocortisone to mothers just prior to "elective, repeat, cesarean section" was followed by an increase in the levels of 17-hydroxycorticosteroids (17-OH-CS) in cord plasma. It was also observed that in cases of vaginal delivery as well as in cases of cesarean section, with or without infusion of corticotropin or hydrocortisone, the concentrations of 17-OH-CS in the cord plasma were one-half to one-fifth of those in the mother. This suggests that hydrocortisone is transferred from the mother to the baby but in a definite proportion.

In order to clarify the metabolism of hydrocortisone during pregnancy, near term, additional work was carried out, using 4-C^{14}-hydrocortisone. It was confirmed that hydrocortisone can pass the "placental barrier," the amount of radioactive steroid crossing this barrier representing approximately 2% to 3% of the dose administered to the mother. The metabolism of 4-C^{14}-hydrocortisone in the mother was found to be markedly altered. In plasma, the half-life of "free" radioactivity was twice that observed in control subjects and the radioactivity released by β-glucuronidase hydrolysis was less than normal. The total amount of urinary radioactivity excreted was diminished, with an increase in the free fraction and a decrease in the glucuronoside fraction. The data suggest that the increase in plasma "free" 17-OH-CS observed near term is related to a slower rate of catabolism of hydrocortisone rather than to a more rapid rate of its production.

Discussion

JOSEPH DANCIS, New York: I should like to comment upon one aspect of the study that has just been presented, just to highlight some of the difficulties in the interpretation of placental function from maternal and fetal blood levels. When C^{14}-estradiol is injected into the pregnant guinea pig and the radioactivity in the fetal blood is determined a few minutes later, most of it is water-soluble. This suggests preferential transfer of the conjugated forms of estradiol. However, we have since discovered that the fetus is capable of converting the lipid-soluble, "free" estradiol to the water-soluble form, thus simulating preferential transfer. More recently we have modified the experimental technique, replacing the fetus by an artificial circulation. We now find that the placenta is readily permeable to the lipid-soluble forms but relatively impermeable to the glucuronide.

The situation is actually far more complex than this. It is obvious that, with materials that are rapidly metabolized by mother and fetus, one must be careful not to oversimplify the interpretation of data.

ROBERT A. ULSTROM, Minneapolis: My remarks are of the same nature as those of Dr. Dancis, concerning the specificity of what is actually being measured. The measurement of C^{14} after injection of labeled hydrocortisone, when measured as the extract of plasma, does allow room for the possibility of measuring certain other newly formed compounds. The question of breaking the steroid nucleus is not one that is pertinent, but certainly

* By invitation.

the possibility that side-chain reductions could be occurring before conjugation is something that must be considered. One is not entirely justified in defining the radioactive counts in this particular situation as hydrocortisone in either the mother or the baby.

The demonstration that a steroid is crossing the placenta under what would be considered very close to natural circumstances is a very important one. Dr. Migeon has previously shown that when unlabeled steroid was given or the mother's adrenal was stimulated that this would occur at increased maternal blood levels of steroid.

I believe that this paper is important because we now can be assured that the steroid is being transferred without an artificial elevation of the maternal level. The question of how much of this radioactivity is actually in hydrocortisone is one that needs further definition.

The question of how much hydrocortisone is in the infant which has come from the mother, I believe, also remains to be answered. One of the unanswered problems of adrenal physiology in the newborn infant is whether the newborn infant has any adrenal activity in utero and shortly after birth. Klein's demonstration of blood level changes would lead us to think in one direction; those of Kelley, in another. I think this paper is a step toward an answer, but the amount of hydrocortisone in the baby which is contributed by the fetal adrenal and the amount which is contributed by maternal transfer across the placenta, relatively speaking, are not strictly defined here.

VINCENT KELLEY, Salt Lake City: I think that one other factor which should be considered here is a possible difference in the rate of metabolism of the steroid by the fetal organism as opposed to that by the mother. I have no idea what the rate of metabolism of hydrocortisone by the fetus might be. However, I do know that in a newborn infant the half-life of hydrocortisone is roughly one-third of that in the normal adult. If the half-life of hydrocortisone in the mother is prolonged and if the half-life in the fetus is anything like that in the newborn infant there would be a five- or sixfold differential in these rates of metabolism; this conceivably could help explain the apparent distribution differential between the mother's blood and the cord blood. I wonder whether this has been taken into consideration or whether the speaker would care to comment about this.

EDNA SOBEL, New York: I wonder if Dr. Stempfel would tell us about the amount of radioactivity which was given to the mother and how much of this he thinks might apply to the infant. This is always a problem in studies of human physiology, which makes some of us wonder whether animals might be better experimental subjects. I am sure the authors have given thought to this problem, but I would like to know the dose of radioactivity.

CLAUDE J. MIGEON, Baltimore: I wonder if Dr. Stempfel will excuse me if I try to answer Dr. Edna Sobel's question on the safety problem. We have been concerned ourselves about this matter.

In a preliminary study with nonisotopic hydrocortisone we determined that approximately 3% of a dose given to the mother would cross the "placental barrier" to the baby. We also found that the rate of excretion of hydrocortisone by the newborn was somewhat similar to that obtained in adults. From these observations, we calculated that the amount of radiation received by the baby would be about one-tenth of that which he would receive from a routine chest x-ray. Of course, we realize that even one x-ray should be avoided whenever possible, but it was thought that the information obtained from such studies might be of clinical interest. Furthermore, it was our purpose to study as few subjects as possible.

ROBERT S. STEMPFEL JR., Baltimore: We have debated the problems expressed by Drs. Dancis and Ulstrom. Although we have no definitive answers, a further step in the identification of radiocorticoids transferred across the placenta was accomplished by means of paper chromatography of the "free" extracts of maternal and cord plasma. About 40% to 50% of the free radioactivity was eluted from the hydrocortisone area, while the remainder could be accounted for by dihydro and tetrahydro derivatives of hydrocortisone. Of perhaps greater interest were the urinary findings. After the removal of the chloroform-extractable ("free") fraction and after the removal of those fractions extracted after hydrolysis with β-glucuronidase, continuous ether extraction for 48 hours at pH 0.8, and strong acid hydrolysis, we still found a great deal of unaccounted-for radioactivity in the residue. This was more pronounced in the infant's than in the mother's urine. It is possible, therefore, that the newborn infant has other modes of conjugation.

When we speak of a lack of fetal hydrocortisone production, we do so only by inference, as we studied only the cord blood. Perhaps Dr. Dunham's suggestion that we take our problems to the marsupial is valid here.

Dr. Kelley's interesting question concerning rates of metabolism of hydrocortisone in the mother and fetus has been a constant consideration. It would seem unlikely, however, that a very rapid excretion of corticoids by the fetus could account for the maternal-cord ratios observed, since these ratios were relatively constant except where the maternal values were lower and the ratio had a tendency to be smaller. When the plasma glucuronoside fractions were determined the maternal-cord ratios fell within the same range as the "free" fractions. This would also favor a true concentration gradient

rather than a difference in rate of conjugation to explain the lower fetal "free" values.

To further augment Dr. Migeon's answer to Dr. Sobel's question, I should like to say that the exact amount of C^{14} administered to each mother was 1 μc.

In Vitro Biogenesis of Steroids by the Human Fetal Adrenal. JONATHAN T. LANMAN, SAMUEL SOLOMON,* JOHN LIND,* and SEYMOUR LIEBERMAN,* New York.

The function of the fetal zone of the human adrenal gland is not known. It is uncertain whether this tissue participates in the biogenesis of the steroid hormones which have been reported in extracts of whole fetal adrenals. Experiments were carried out to determine whether fetal zone tissue can convert progesterone into adrenal hormones. Adrenal glands obtained from legally aborted normal fetuses were frozen and dissected into an inner portion (fetal zone) and an outer portion (fetal zone plus adult-type cortex). Histological examination of the inner tissue fraction revealed occasional bits of adult-type cells in amounts which we believe were too small to influence these results. Each portion was homogenized and incubated with 3.6 mg. progesterone-4-C^{14} (25.8×10^6 cpm [counts per minute]) and 5 mg. each of nonradioactive androstenedione and 17-OH-progesterone. Following incubation, neutral residues were prepared and, after preliminary fractionation on silica gel, were chromatographed on paper, with use of the Zaffaroni and Savard type of systems. The individual zones were located on the strips by radioautography and by scanning under ultraviolet light, and their positions were compared with those of standard steroids.

Both outer and inner portions of the fetal adrenals converted about 90% of the radioactive progresterone into a number of radioactive compounds. After paper chromatography in two different systems, the zones corresponding to the two carrier compounds were radioactive. After elution, crystalline androstenedione was recovered and found after extensive purification to have a specific activity of 308,000 cpm per milligram (inner tissue portion) and 247,000 cpm per milligram (outer tissue portion), indicating a conversion of approximately 5% of the radioactive progesterone to androstenedione. The zones on paper corresponding to 17-OH-progesterone were eluted, and carrier steroid was added. After purification, the 17-OH-progesterone was radioactive, and radiochemical homogeneity was established.

In addition, at least three fractions corresponding in mobility to $C_{21}O_5$ compounds, two fractions corresponding to $C_{21}O_4$ compounds, and one other fraction moving with $C_{19}O_3$ compounds have been isolated. The radioactivity of each fraction coincided on the paper strip with the ultraviolet-absorbing material. Attempts to establish the identity and radiochemical homogeneity of certain of these fractions are currently under way.

* By invitation.

Discussion

ROBERT A. ULSTROM, Minneapolis: I think that enzymatic evidence, of which this is really representative, is going to lead the way to much better understanding of the function of the fetal adrenal cortex and the role of the adrenal cortex during the prenatal and postnatal periods.

I was particularly interested in the finding of a spot which corresponded with cortisone (Compound E) in the chromatograms, although Dr. Lanman indicated that he had not provided the most rigorous proof of its identity. This interested me because in the urines of newborn infants we have found something which we have not final identity of, but which we believe is Compound E, in an amount which has surprised us. This gives us a little more confidence that we may actually be looking at Compound E. I hope Dr. Lanman will have further opportunity to prove what this spot is by more rigorous methods.

A. M. DIGEORGE, Philadelphia: I wonder if Dr. Lanman has any evidence that there might be a difference in the biosynthesis of these compounds by the fetal cortex at different times throughout fetal life. What do you suspect is going on at term? Is synthesis just as active at this stage of fetal life as it is during the third to fifth months of fetal life?

JUDSON J. VAN WYK, Chapel Hill, N. C.: I should like to ask Dr. Lanman whether he has attempted to stimulate further synthesis of steroids by in vitro incubation with corticotropin. Whole adrenal slices can be stimulated to secrete steroids if cholesterol is used as the precursor. A modification of this technique might shed some light on which trophic hormone is active on this zone.

JONATHAN T. LANMAN, New York: Regarding the radioactive band occupying the position of cortisone on these paper chromatograms, I would not offer this as proof or even very good evidence of the presence or absence of cortisone, and I would much prefer to wait elution of these bands and further chemical identification.

We have not in previous studies had evidence of cortisone production in the fetal glands, although we believe we have been able to establish the synthesis of Compound F by the glands of the neonatal infant.

In answer to Dr. DiGeorge's question, we have no information as to biosynthesis at different times of fetal life. The gland is proportionately largest at about the gestational age when these fetuses are aborted. If size is an indication of activity, which it

may well not be, then biosynthesis would be most active in the younger fetuses.

In answer to Dr. Van Wyk, with regard to the addition of corticotropin to these preparations, it is my impression that corticotropin is of no stimulating value in homogenates, although it is in perfused glands and may be to some extent in tissue slices. These were homogenates, and I do not believe that corticotropin would stimulate them.

Histochemistry of Experimental Nephritis and Nephrosis With and Without Cortisone Treatment. MAX WACHSTEIN* and KURT LANGE,* New York Medical College. Introduced by D. GRIBETZ.

Experimental nephritis and nephrosis were produced in rabbits by the administration of rabbit kidney localizing duck sera. Animals were studied for antibodies against duck serum, serum complement, blood urea nitrogen, total serum protein, albumin-globulin ratios, and cholesterol levels, and the urine was studied for proteinuria and microscopic constituents. A second group of animals were treated with daily injections of large amounts of cortisone after the initial injection of the rabbit kidney localizing duck serum. Six animals were injected with Evans blue, 24 hours before they were killed. Animals were killed after various staggered intervals, and sections for microscopic studies were stained with routine and histochemical techniques.

In the group not treated with cortisone there is a latent period of five to seven days in which no clinical signs or symptoms of glomerulonephritis or nephrosis can be detected. Antibodies to duck serum are not present and complement remains normal. The glomerular capillaries, however, show a moderate proliferation of endothelial cells, without evidence of increased transudation. Simultaneously with the appearance of clinical disease, i. e., appearance of antibodies against duck serum, fall in serum complement, and massive proteinuria, alterations in the glomeruli become very prominent. There is a marked increase of cells in the glomerular tufts and deposition of intensely PAS-positive material within capillary lumina and in Bowman capsules. Many tubules contain PAS-positive casts. During the latent period the tubular cells, as in the normal animal, do not contain Evans blue or contain only very small amounts of it. However, the tubular cells become deeply stained by the dye upon the appearance of proteinuria; at the same time, the proximal convoluted tubules become filled with hyaline PAS-positive droplets, presumably reabsorbed protein. These droplets disappear after five or six days, although the proteinuria may continue to be as severe as before. With the disappearance of the droplets, degenerative changes in the tubules become manifest. There is marked cytoplasmic vacuolization and atrophy of tubular cells. Various histochemical reactions show no significant deviations from normal during the first five to six days of the proteinuria. Six to eight days after the onset of clinical disease, however, marked depression of certain enzymatic activities is seen in the proximal convoluted tubules. Such depression is particularly noticeable in sections stained for alkaline phosphatase, glucose-6-phosphatase, succinic dehydrogenase, and DPN diaphorase activity.

In animals which survived for long periods of time (40 to 50 days), some tubules revealed normal staining patterns while the majority showed no evidence of histochemically demonstrable enzyme activities.

In previous studies it had been shown that large doses of cortisone can prevent or minimize the development of abnormal function, i. e., clinical and laboratory findings. In this study the functional changes, on the one hand, and the morphologic and histochemical changes, on the other, were graded independently. A good correlation has been shown between protection of function by cortisone and normal or near normal morphology and histochemistry.

Discussion

HENRY L. BARNETT, New York: In order to understand the relationship, if any, between these various experimental diseases and the disease that we see in children, I should like to ask a few questions about the disease that the authors have produced in rabbits. I should like to know, for example, whether the blood pressure was measured and if it was elevated and about the degree of hematuria.

In addition, I should like to ask whether they have looked at capillaries elsewhere in the body, and, if so, if there were changes.

JOHN M. CRAIG, Boston: The problem of the development of the protein droplets in the renal tubules has been one of considerable interest to me. One wonders how much these are related to the proteinuria that occurs in these animals. It appears that there is a good deal of coincident overlapping of the atrophy of tubular cells and the proteinuria, and it may be difficult for Dr. Wachstein to separate out the role of proteinuria and the role of decreased blood supply in the development of the droplets. I wonder if he knows whether the early changes in the tubules are solely due to proteinuria.

VINCENT KELLEY, Salt Lake City: I should like to ask two questions with relation to possible clinical implications of this study. 1. How long after the nephrotoxic serum has been given can the cortisone be given and still be effective?

2. The other question has to do with the size dose of cortisone necessary to protect the kidneys. I

* By invitation.

noticed that the dose of cortisone indicated on one slide was 20 mg. per kilogram per day. Does the dose have to be this high in order to protect the kidneys? This would be an impractical dose to use in the human subject. Can a smaller dose, like 5 or 10 mg. per kilogram per day, be employed.

WALTER HEYMANN, Cleveland: The disease in rabbits induced by duck serum is less well known in details of blood chemistry deviations than the one in rats. Could Dr. Wachstein give us some information on the severity of the hyperlipemia observed in these rabbits?

HERMAN W. SPATER, New York: I should like to know whether PAS-positive droplets can be seen in the normal rabbit kidney tubule and whether or not the normal rabbit has a minimal physiologic proteinuria.

MAX WACHSTEIN, New York: In answer to Dr. Barnett's question, I do not think that blood pressures were measured, but I should like to ask Dr. Lange about that. All these animals had proteinuria, but the capillaries, as far as we have determined in various other organs, do not show very much.

I might answer Dr. Heymann's comment at the same time. This disease has been, from a pathological point of view, fairly well studied by Masugi, his pupils, and several German authors. Most of the papers are about 20 years old, and much of the basic pathology that we have shown here has already been described previously. The only findings these older authors did not pay much attention to were the tubular changes. They were so impressed by the glomerular changes that they mentioned only that an occasional one of these animals had hyaline droplets in some tubules.

This brings me to the question of Dr. Craig, and, of course, that is a very intriguing question, which we are very much interested in. There are two interpretations—of course, there are probably many more, but two come to one's mind right away: Is it possible that the tremendous amount of protein droplets damages the tubules so much that in consequence a rapid tubular atrophy is produced? On the other hand, tubular atrophy in this very violent disease may occur very rapidly, dependent upon the glomerular changes.

As far as the large doses of cortisone that were given are concerned, the dosage in some of the animals was less than 30 mg. Some of them got only 5 mg. daily, and usually the treatment was started simultaneously with the giving of the damaging serum. There is little stainable fat present in damaged tubules. This is in agreement with the findings reported in the literature. The animals showed regularly increases in serum cholesterol.

Dr. Spater, I would say that it is very difficult to be quite sure that in the normal rabbit kidney PAS-positive granules are present. If they are, they are certainly very small and only occasionally present.

KURT LANGE, New York: In answer to Dr. Barnett's question, I can state that red cells appear in the urine with large amounts of immune sera, while small amounts will only cause proteinuria.

In answer to Dr. Kelley's question, I wish to state that we have tried to protect animals with cortisone in doses varying between 2 mg. to 30 mg. per kilogram per day. Much better protection is naturally obtained with the higher doses, since they suppress antibody formation more effectively, but you can note a significant protection starting with a dose of 5 mg. per kilogram per day.

I wish to state, however, that doses of 20 to 30 mg. per kilogram per day are comparable to the doses we like to give to children. This high dosage may be the reason why our results are in general better than those obtained by others using much smaller doses.

Dr. Heymann asked whether hyperlipemia can be found in these rabbits. The answer is yes. The normal cholesterol level in rabbits varies between 40 and 90 mg. per 100 cc. In animals having a nephrotic syndrome for several days, values between 200 and 300 mg. per 100 cc. are common.

Renal Tubular Lesions in Hyperelectrolytemia.

JAMES B. AREY and HELEN REARDON, Temple.

During the period 1952-1956 postmortem examinations have been performed on 10 patients known to have hypernatremia. In two infants primary renal disease, i. e., renal hypoplasia with pyelonephritis and multiple renal abscesses with intrarenal venous thrombi, was present. Among the remaining eight patients, in whom no significant preexisting renal disease was present, definite renal cortical tubular dilatation was present in four. These four infants were all admitted with a diagnosis of gastroenteritis, with periods of dehydration varying from two to five days.

Review of sections of the kidneys of 297 infants and children dying during this same period of time has revealed comparable renal cortical tubular dilatation in 26 patients in whom evidence of hyperelectrolytemia was lacking; the lesion thus is not pathognomonic of this condition. Nevertheless, it has occurred with sufficient frequency so as to be a valuable but not infallible means of suggesting the presence of hyperelectrolytemia on the basis of morphologic alterations.

Discussion

WALLACE W. MCCRORY, Philadelphia: I wonder whether Dr. Arey made any attempt to evaluate the treatment prior to death in some of these children. I think it is not unlikely that children admitted

critically dehydrated may have received infusions terminally. It is not inconceivable that in a child who is going to die and who is receiving parenteral fluid therapy an anuric kidney might show dilatation, whereas this might not be the case without therapy. Does he have information on this point?

MALCOLM A. HOLLIDAY, Pittsburgh: I was wondering if some of these changes, i.e., dilalation, might be explained by the very simple mechanism of the plug demonstrated in the terminal collecting tubule. The lack of regeneration would suggest that this is not a potassium-deficiency lesion. Further, the lesion in potassium deficiency in the collecting tubule at least appears to develop rather late, requiring a state of deficiency longer than the illnesses noted here.

I should like to ask Dr. Arey if these children had any evidence of thrombosis. I have been impressed on occasion with the development of thrombosis in association with hyperelectrolytemia.

LAURENCE FINBERG, Baltimore: I should like to confirm at least part of Dr. Arey's study, insofar as the experimental animal is concerned. In kittens that we have made hypernatremic we have seen lesions of the tubule similar to his. Unfortunately, in littermates which we stimply dehydrate without causing hypernatremia we see similar lesions.

I wonder if perhaps the common denominator in his study might be simple dehydration. Does he have information on the hydration of his control cases?

ALLAN M. BUTLER, Boston: I should like to ask if there are any electrocardiograms on these patients.

MITCHELL I. RUBIN, Buffalo: It is of interest that you found tubular changes in dehydrated infants, since in a group of severely dehydrated infants with diarrheal disease we found a loss of tubular concentration capacity. The dilated tubules you show are similar to what is seen in pyelonephritis and could result from tubular blockage in a more distal segment.

HENRY L. BARNETT, New York: I should like to ask whether these lesions bear any resemblance to the lesions that were observed both clinically and experimentally with the administration of 50% sucrose and hypertonic solutions a number of years ago.

HELEN REARDON, Haverford, Pa.: In reference to the question of Dr. McCrory, there was no uniform method of fluid therapy, owing to several factors: (1) the various causes of illness and (2) the lack of recognition of hypernatremia in four patients prior to death.

One of the patients with gastroenteritis received adequate fluid and electrolyte therapy. The three other patients received excessive sodium intake by present criteria.

Of the six remaining patients, all with different diagnoses, two may have received excessive sodium therapy. The other four infants had excessive water losses, which were probably not covered by adequate fluid administration.

JAMES B. AREY, Narberth, Pa.: Dr. Holliday, certainly the possibility that plugs of material in the terminal collecting tubules are responsible for the tubular dilatation which was noted must be considered. Such acidophilic casts were noted in the terminal collecting tubules in three of the four patients with hyperelectrolytemia in whom cortical tubular dilatation was present, and it is, of course, possible that they might have been missed in the fourth patient. However, it seems unlikely that such casts would have been overlooked in a considerably larger group of patients (26), in whom comparable renal cortical tubular dilatation was present in the absence of known hyperelectrolytemia. The majority of these 26 patients had no demonstrable plugs of acidophilic material in the terminal collecting tubules. Thus, although dilatation secondary to such an obstruction remains a possibility, it would appear to be an unlikely one.

Evidence of thromboses was present in only one patient with hyperelectrolytemia. This infant had severe pyelonephritis with multiple renal vein thromboses.

Dr. Finberg, I am very happy to hear that you have been able to produce a comparable lesion experimentally in kittens. Dr. Reardon, Dr. Vega, and I have attempted to produce this lesion in a small number of dogs in whom hyperelectrolytemia of short duration had been induced, but to date we have been quite unsuccessful.

It certainly would seem possible that dehydration may be the factor responsible for the production of this lesion, but our data are insufficient to prove or disprove this fact.

Dr. Butler, we do not have electrocardiographs on most of these patients, many of whom died within 24 hours after admission to the hospital. Histologic sections of the myocardium did not reveal the morphologic changes associated with hypokalemia, but this admittedly is not an exact method by which this diagnosis can be excluded.

Dr. Rubin, thank you for your comments concerning the loss of tubular function. With respect to the dilatation of tubules associated with pyelonephritis, it is possible that this may be in part the result of obstruction by casts. However, it is my impression that renal tubular dilatation associated with pyelonephritis is primarily the result of hypertrophy of residual nephrons which have not been destroyed by the inflammatory process.

Dr. Barnett, I believe that the lesion described in these patients is not the same as that associated with the administration of 50% sucrose. The

latter is associated with severe hydropic degeneration of the renal tubular epithelium.

An Epidemic of Aseptic Meningitis Due to an Enteric Virus Related to the ECHO Virus Group. DAVID T. KARZON, ALMEN L. BARRON,* and Beatrice F. Pollock,* Buffalo.

A combined clinical, epidemiological, and viral study was made of patients and their household contacts during an epidemic of aseptic meningitis in Buffalo in the summer of 1956. Approximately 500 persons were studied. A total of 222 nonpoliovirus enteric cytopathogenic agents were recovered in monkey kidney tissue culture from stools, throats, and cerebrospinal fluids (CSF). The predominant virus type recovered, represented by the prototype designated in this study as the Shropshire virus, appears to be closely related to the ECHO (enteric cytopathogenic human orphan) virus group. The virus recovery rate, to date, in aseptic meningitis patients has been as follows: stool, 52%; throat swab, 41%, and CSF, 22%. From household associates of patients the recovery rate has been as follows: stool, 21%, and throat swab, 6%. From patients with diagnoses other than aseptic meningitis, admitted during the epidemic period to the contagious service of the Buffalo Children's Hospital, only two agents were recovered from the 159 specimens examined. Poliovirus was consistently recovered from the relatively few patients with paralytic poliomyelitis and their household contacts during the aseptic meningitis epidemic. In patients with aseptic meningitis and their household contracts, the presence of a possible viremia during the acute stage and the persistence of virus in the stool, pharynx, and CSF during convalescence were also studied.

The epidemic of aseptic meningitis was confined largely to the Negro population of downtown Buffalo. This is in marked contrast to the outbreak of aseptic meningitis due to ECHO 6 in western New York in 1955, when the disease was uncommon in Negroes and was not localized in any area. The mild transient muscle weakness and the changes in deep and superficial reflexes observed in the 1955 ECHO 6 infection were less prominent in the 1956 disease.

The Shropshire virus itself appeared to be unusual in several properties. The agent was not readily identifiable as one of the 14 known ECHO serotypes. However, it was found to share antigens in common with ECHO Types 1, 8, and 13 and most closely resembled ECHO Type 4. The agent was not passageable in suckling mice. The Shropshire virus was found to be a conspicuously poor antigen, as evidenced by the extremely low titer of neutralizing antibody produced in patients following infection, and it was also a poor antigen experimentally in the rabbit and chicken. Shropshire rabbit antiserum, although of low homologous titer, neutralized to some extent enteric viruses outside the ECHO group, namely, certain Coxsackie viruses and poliovirus Type 2. The implications of the antigenic interrelationships of the Shropshire agent with other enteric viruses were discussed.

Discussion

ROBERT WARD, New York: Dr. Wenner, I believe, reported an epidemic of aseptic meningitis caused by ECHO Type 4. I wonder if Dr. Karzon had any opportunity to see what was the relationship of his Shropshire virus to Wenner's Type 4.

EDWARD C. CURREN JR., Chapel Hill, N. C.: This is an impressive report and adds further information on the capacity of members of this group of viruses to cause human disease. Dr. Karzon's evidence is convincing that the "Shropshire" virus was causally related to the outbreak which he described. The recovery of the virus from the spinal fluid of representative patients is especially important in this respect. I am interested to know whether he can tell us about the relationship of this virus to the one that Dr. Wenner encountered and also whether he will tell us more about other clinical manifestations of illness associated with the viruses which he encountered in the two outbreaks in 1955 and 1956.

C. HENRY KEMPE, Denver: I should like to add to Dr. Ward's and Dr. Curnen's comments only to say that it may well be that we are getting close to finding stem viruses for the ECHO group. This particular agent has neutralization to Coxsackie A and to Coxsackie B and to polio Type 2 viruses. Dr. Karzon's paper also makes a liar of the term ECHO, because more and more we are finding that these are not orphans at all and that these enteric organisms are indeed capable of being parents of some pretty juicy diseases.

HEINZ F. EICHENWALD, New York: As one who has been fighting the battle of the ECHO's for the past year, I am really lost in admiration for the beautiful way in which Dr. Karzon attacked this difficult and intricate problem.

I should like to ask him two questions. An ECHO is supposed to be a virus to which antibodies are found in normal human γ-globulin. I was wondering, in the light of the very poor antibody response to this agent, whether antibodies to this agent are present in normal human γ-globulin and whether in Dr. Wenner's Type 4 outbreak the antibody rises were of the same low order of magnitude as they were in this particular episode.

DAVID T. KARZON, Buffalo: I can group my comments into two sections: first, the clinical illness; secondly the nature of the virus.

The aseptic meningitis associated with Shropshire virus was a mild disease and resembled in all

* By invitation

respects the illness that all clinicians would agree has been called nonparalytic poliomyelitis. I do not think that I need run through the symptoms and signs. The interesting features of the clinical disease are the extent of neuromuscular findings in the acute stage and the extent of residua which may occur in the wake of infection with this group of viruses.

With the ECHO 6 disease in 1955, approximately 50% of the cases had mild neuromuscular involvement. This consisted of depression of superficial and deep reflexes, which was usually transient but sometimes persisted, and very mild but definite weakness of certain muscles. Characteristically, they were axial muscles—anterior neck, back, the shoulder girdle, or the pelvis—rarely a peripheral muscle. We are now engaged in a follow-up study to see what, if any, damage has been left. It is certainly minimal. In 1956 the disease which was caused by ECHO 4 was milder. All the neuromuscular findings I have described occurred, but in a lower incidence, despite the fact that we were alerted to look for them.

The relationship of the Shropshire agent to other enteric viruses has intrigued us very much, and I personally think that it may be more important than simply associating another disease with another virus.

We have had an opportunity to compare the Shropshire virus with Dr. Wenner's field strains of ECHO 4, and this proves very interesting. It points up a trap in this group of diseases. When one uses for the serological identification of an ECHO virus the prototype strain, which is all very well and proper, one is simply using a representative of a serotype which is very variable. That is, the prototype is simply one sample. Other samples of wild strains of that type would look slightly different antigenically, each from the other. So that when we compared various isolates from our outbreak with the prototype ECHO 4 and with Dr. Wenner's samples of ECHO 4, we were able to find every gradation of identity and dissimilarity between our strains and ECHO 4. It would look as if we have shown extensive variance within a single serotype.

Dr. Kempe brought up a very interesting point. We have available a great deal of material on which not only to do taxonomic studies but to try to figure out the phylogenetic interrelationships of these organisms. So far, our working assumption is that the cross neutralizations are real and are dependent on antigen-antibody relationships rather than on nonspecific factors.

Now, the significance of this to human illness conceivably could be important in that one's serological experience with a variety of enteric agents may affect the clinical response to future enteric agents.

In reply to Dr. Eichenwald's question concerning the antibody level against the Shropshire agents in γ-globulin, it is not present against several strains in a titer as low as 1:4. This does not surprise us, because of the poor antibody response to this antigen in man and experimental animals.

I have no data comparing antibody rises with Dr. Wenner's patients.

Acid Intermediates of Metabolism in Sweat in Cystic Fibrosis. GORDON E. GIBBS, VIOLET M. WILDER,* JOHN B. MCANDREW,* and DANIEL G. BOHI,* Nebraska.

A search has been made for evidence of a hypothetical defect in sweat gland metabolism responsible for the electrolyte abnormality of sweat in cystic fibrosis. Sweat was collected in gauze and recovered undiluted by centrifugation.

Lactate in sweat was found in concentration of 1.3±0.4 (S. D.) mg. per milliliter in 9 patients with fibrocystic disease and 1.5±0.6 mg. per milliliter in 16 controls.

Pyruvate was found in concentration of 79±18μg. per milliliter in 8 patients with fibrocystic disease and 64±41μg. per milliliter in 13 controls.

"Citrate" (Krog method) appeared to be in concentration of 31±13μg. per milliliter in 13 patients with fibrocystic disease and 13±3μg. per milliliter in 20 controls. While this difference is statistically highly significant, the biochemical meaning of the result remains unclear. Some of the control level is probably due to lactate.

Malate was found to be present at a concentration of 22±5μg. per milliliter in six patients with fibrocystic disease and 25±17μg. per milliliter in seven controls.

In summary, no significant abnormality occurred in lactic, pyruvic, and malic acid concentrations of sweat in cystic fibrosis of the pancreas. An elevated level of apparent citric acid occurred and provides a clue for further investigation.

Discussion

ROBERT KLEIN, Pittsburgh: I think these are very interesting data, and I realize that Dr. Gibbs is just beginning his studies, but I should like to put in a plea to him and to others that have been working with sweat that actual volume of sweat be considered. I think that Cooke is the only one that has measured this at all, and I am not sure that his controls were adequate; if I am correct, they were siblings of the patients. But Schwartz has shown, for instance, that the concentration of sodium in sweat is proportional to the volume of sweat excreted per minute. Once a threshhold was overcome this rose steadily, and so I think that this is a very important factor. It would involve, I should think, collecting under some

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standard situation, and I am not sure that we can control a hot room or blankets and heating pad adequately.

ROBERT E. COOKE, Baltimore: The question of whether or not the citrate or citric-like compound was the result of materials on the skin I think could be settled readily by the use of the washout technique which Schwartz and others have used, in which preliminary sweating several times is used, with washing the skin and discarding those samples, and then the final material is collected after the washout period. I think this would get rid of the question of skin desquamation. Potassium levels in the skin in the sweat, for example, will fall considerably and then level off with prolonged washout.

For the sake of general interest and saving of time, it is worth pointing out that the concentration of amino acids in the sweat of these persons is the same as in normal persons. This is with a controlled rate of sweating. Two-dimensional chromatograms, both quantitatively and qualitatively, are similar as far as amino acid patterns in persons with cystitis and normal persons are concerned.

I think it is worth commenting that materials which appear in the sweat may not necessarily be metabolites of the sweat gland. I think that this is an assumption that has not been proven by any means whatsoever. Lactic acid levels are high, and there is contradictory evidence as to whether the lactate is being transported across the cell or whether this is actually lactate coming from the cell itself.

GORDON GIBBS, Omaha: The discussants have been very kind and have not asked me any of the embarrassing questions they could have. I appreciate, very much, the suggestions they have made.

We have considered that in a large enough number of cases the variation in rate of sweating would cancel out in the patients with fibrocystic disease and on the controls. In the citrate, where we seem to have a nibble of something, our next step is to relate this level to the rate of sweating which obtains in the particular case.

Urinary Hexuronic Acid Determinations in Prematures, Newborns, and Young Infants. PERPETUA S. LACSON* and WILLIAM J. WATERS, Syracuse, N. Y.

Following the report of Schmid that direct bilirubin is a bilirubin-glucuronide, hexuronic acid excretion in the urine was determined as an indirect measure of the functional capacity of the liver for conjugation. (The assumption is made that the major component of the hexuronic acids in the urine is glucuronic acid.)

Twenty-four hour urine collections were obtained, and measurements of hexuronic acids were made on 1 cc. aliquots according to the method of Z. Dische. Determinations were made from specimens from premature infants, normal newborn infants, infants with hemolytic disease of the newborn, and young infants with various disease states.

The values ranged from 0.1 to 0.3 mg. per cubic centimeter in normal newborn and premature infants without disease, with a total 24-hour excretion of 5 to 35 mg. during the early newborn period. The lowest values were obtained in premature infants. Higher values were found in hemolytic disease of the newborn, particularly in those infants who required replacement transfusions. However, the response was variable, ranging from 0.1 to 1.2 mg. per cubic centimeter and 8 to 89 mg. total per 24 hours. The high values appeared to correlate with the degree of bilirubinemia. Of interest is the fact in this group that one patient showed urobilinogen in the urine as early as the second day, suggesting that conjugation had taken place.

Determinations from older infants with various disease states were also performed. The highest values of glucuronic acid in this series were found in two patients with biliary atresia, 0.65 mg. and 1.5 mg. per cubic centimeter and a total of 136.5 and 408 mg., respectively, in 24 hours.

Determinations were done on normal adults and ranged from 0.27 to 0.83 mg. per cubic centimeter and the total from 374 to 913 mg. These values are not dissimilar to those reported in the literature.

The mean values in normal newborn infants were 0.17 mg. per cubic centimeter and 13 mg. total in 24 hours.; in premature infants, 0.12 mg. per cubic centimeter and 12.9 mg. total in 24 hours; in mild hemolytic disease of the newborn, 0.27 mg. per cubic centimeter and 14.6 mg. total in 24 hours; in severe hemolytic disease of the newborn, 0.42 mg. per cubic centimeter and 31 mg. total per 24 hours.

Our data show that total urinary glucuronic acid present in normal newborn and premature infants is comparatively lower than in older infants and adults. The response to bilirubinemia is variable, but in general it is associated with an increase in urinary glucuronic acid excretion.

It is further suggested that the conjugation mechanism is present in the newborn infant but seems to be relatively less effective, at least within the first week of life.

Studies on the Neonatal Development of the Glucuronide Conjugating System. AUDREY K. BROWN,* with the assistance of HERBERT BURNETT, Wayne. Introduced by WOLF W. ZUELZER.

It has long been recognized that the majority of newborn infants exhibit jaundice during the

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first few days of life, due chiefly to the accumulation of "indirect" bilirubin. It has been postulated that this accumulation is due to a defect in the metabolism of bilirubin rather than to increased production of bilirubin. Recently it has been shown that the "direct-reacting," water-soluble bilirubin in jaundiced serum and urine and in normal bile is conjugated with glucuronic acid, whereas "indirect-reacting" bilirubin is unconjugated.

Enzymatic synthesis of bilirubin glucuronide has been demonstrated recently by Schmid and Axelrod, utilizing liver microsomes. The formation of bilirubin glucuronide seems to involve the same system as does conjugation of substances as *o*-aminophenol or phenolphthalein with glucuronic acid. This reaction is catalyzed by an enzyme system in microsomes of mammalian liver of several species and requires uridine diphosphate glucuronic acid (UDPGA) as the glucuronyl donor. This reaction would appear to proceed as follows:

$$\text{UDPGA} + \text{bilirubin} \ (\text{"indirect"}) \xrightarrow[\text{transferase}]{\text{glucuronyl}} \text{bilirubin glucuronide} \ (\text{"direct"}) + \text{UDP}$$

In our investigations, microsomes of liver from fetal, newborn, and adult guinea pigs were used to demonstrate the development of the glucuronide conjugating system. The data indicate that when *o*-aminophenol and phenolphthalein are used as the substances to be conjugated, liver microsomal activity is absent in very young fetuses, is very low in fetuses almost at term, and increases during the first week of life in the newborn animal. When bilirubin was used as the subrate, newborn and fetal microsomes showed virtual inability to form the glucuronide as compared with the adult microsomes. Investigation was also made of the in vitro conjugation of sulfobromophthalein (Bromsulphalein), with use of UDPGA and microsomes from guinea-pig livers. Preliminary data indicate that, at least in vitro, the formation of the glucuronide does take place when microsomes from adult guinea-pig livers are used but is less readily formed with microsomes from newborn livers.

Studies were also made of the system by which

$$\text{UDPG} + \text{DPN} \xrightarrow{\text{UDPG dehydrogenase}} \text{UDPGA} + \text{DPNH}$$

These preliminary investigations indicate that there is decreased activity of the enzyme catalyzing this step when preparations of soluble fraction from livers of newborn animals are used as compared with those from adults.

In order to relate these findings to the situation in the human newborn infant, a study was made of urinary excretion of glucuronides by premature and full-term human infants. This data shows that there is a gradual increase in glucuronide excretion during the first week of life. The initial levels and the rate of increase were lower in the premature infant than in the full-term infant.

These data indicate that there is in the newborn period gradual development of the enzyme system responsible for glucuronide conjugation in the liver. This system is relatively inactive in fetal and newborn guinea-pig livers even when UDPGA is supplied, and this would indicate that a defect lies at the level of transfer of glucuronic acid from UDPGA to the substance to be conjugated. Decreased activity of UDPG dehydrogenase also seems to be present in newborn guinea pigs. The data thus far obtained from study of glucuronide excretion in the human newborn infant indicates indirectly that a similar delay in the development of the glucuronide conjugating system may occur. These findings may help us to interpret the hyperbilirubinemia unrelated to hemolytic disease as well as the variations seen in jaundice associated with hemolytic disease in the newborn.

Discussion

ROBERT A. ALDRICH, Seattle: I should like to make two comments on these presentations. First, I should like to emphasize that for many years careful observers have known that serum bilirubin elevations in the newborn infant cannot usually be correlated with the drop in hemoglobin. Dr. Weech summarized this information in his monograph printed in *Advances in Pediatrics*. The work presented here at this meeting provides us with a mechanism that can be used to explain the well-known facts. The significance of hyperbilirubinemia in the absence of blood-group incompatability now takes on even more interest for biochemists. Second, I want to mention the fact that British and American investigators have presented direct evidence that there are two or more forms of direct-reacting bilirubin. Obviously this may be of importance when assessing the significance of hyperbilirubinemia. Much of the medical literature on hyperbilirubinemia may have to be rewritten subsequently. Finally, it is still apparent that we are far from an explanation for the intracellular changes in the brain that occur in "kernicterus." One might ask why we have the paradox wherein an insoluble form of bilirubin (indirect) seems to readily pass the cell membranes and injure these cells.

ANDREW E. LORINCZ, Chicago: I would like to comment on the assumption made by Drs. Lacson and Waters that the measurement of urinary hexuronic acid represents chiefly glucuronic acid. As reported in an abstract submitted to this society, Dr. Albert Dorfman and I have found an excessive excretion of acid mucopolysaccharides in the Hurler syndrome. The specific mucopolysaccharide

which is most abundant in the urine of these persons has been identified as β-heparin (chondroitinsulfuric acid-B). As is known, the acid mucopolysaccharides are composed of repeating disaccharide units—usually a hexuronic acid, frequently glucuronic acid, (but in β-heparin it is L-iduronic acid), and a hexosamine, which is usually acetylated.

The levels of acid mucopolysaccharides we have obtained in the children above the age of 5 years with the Hurler syndrome have been of the order of magnitude of 0.1 to 0.2 mg. per cubic centimeter of urine. On the basis of the hexuronic acid determination used by the colorimetric method of Dische, this would represent about 37% or 40% hexuronic acid, which is not related to the conjugation of the glucuronic acid with bilirubin.

DR. LILLARD, Kansas City, Kan.: Are we justified in assuming that the urinary glucuronic acid is a true reflection of total glucuronide formation? It is my impression that a considerable amount of it appears in the feces.

SOLOMON A. KAPLAN, Brooklyn: I have a question to ask about the diazo reaction. In view of the fact that you need alcohol and indirect bilirubin to give a blue color with Ehrlich's reagent, is it possible that the free alcohol groups of the glucuronic acid part of the conjugation, are supplying what is necessary for the development of color? In other words, are the free alcohol groups of the glucuronic acid doing what methyl alcohol does in the ordinary determination of bilirubin?

PERPETUA S. LACSON, Syracuse, N. Y.: To Dr. Lorincz' question of whether we were safe in assuming that glucuronic acid was the major component of what we measured, hexuronic acid, we realize that there can be other hexuronic acids in the urine. However, we made baseline determinations in normal newborn infants, and we can not think of any other hexuronic acid that could be present, unless in states of disease.

AUDREY K. BROWN, Detroit: It has become evident that the "direct" reaction of bilirubin is dependent upon the solubility of bilirubin. Since free bilirubin is not water-soluble (except under special conditions), it is not readily diazotized. Conjugated bilirubin or bilirubin glucuronide is water-soluble and is diazotized immediately. We do not know that bilirubin is conjugated only as a glucuronide. There is, of course, the possibility that bilirubin may be conjugated as a sulfate. We have only studied glucuronide conjugating ability.

The differential solubility of free and conjugated bilirubin in various tissues may play a role in the development of kernicterus, as it is evident that kernicterus is associated with the accumulation of the free rather than the conjugated form. We have no experimental evidence concerning the effect of (direct) conjugated bilirubin on brain tissue, as this form of bilirubin is very unstable.

Concerning the interpretation of urinary glucuronic acid determinations, we agree with Dr. Lacson that the carbazole method is specific, with the exception that it would also determine β-heparin. As this has been found in the urine only in Hurler's syndrome and as our babies did not have evidence of this syndrome, we felt we were truly measuring urinary glucuronides.

As to Dr. Kaplan's question as to the nature of the diazo reaction, we would like to know the answer to that question. There are so many things that can potentiate this reaction, such as caffeine and sodium benzoate urea. Conjugated bilirubin is a water-soluble diglucuronide and splits in half as the diazo reaction takes place. Alcohol in the indirect diazo reaction may serve the same function as the glucuronic acid moiety, that is, it may make free bilirubin soluble.

I want to thank Dr. Sass-Kortsak for his kind comments. We were tempted, I must say, having a little prized UDPGA, to administer some of this to newborn infants, even though there is a defect in the transfer of the glucuronic acid to the bilirubin. We have not yet done this. That administration of certain substances may augment this function in the newborn infant is, of course, our great hope. The factors that could hasten the development of the steps leading to glucuronide synthesis in the newborn infant are under investigation.

Urine Composition and Renal Tissue Electrolytes. JOHN D. CRAWFORD, ANTOINETTE PARISI,* PATRICIA MEARA,* and MARY L. TERRY,* Harvard.

Little has been published in confirmation of Wirz' demonstration (*Helvet. physiol. acta* 11:20, 1953) that during antidiuresis there is an osmotic pressure gradient in renal tissue rising progressively from cortex to papilla. The studies to be reported confirm and extend those of Wirz as well as the recent report of Ullrich (*Arch. ges. Physiol.* 262:537, 1956).

Axial cores of kidney tissue have been obtained in vivo from anesthetized rabbits prepared so that the influence of marked changes in urine flow and electrolyte composition could be studied. The tissue cores have been subdivided into sections representative of cortex, outer and inner medulla, and papilla. In company with simultaneously obtained samples of urine and systemic blood plasma, the tissues have been analyzed for water, sodium, potassium, chloride, and urea. Histologic changes have been correlated with the biochemical observations.

The results indicate that at all times there is a gradient with regard to the concentrations of

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sodium and chloride. Values per unit of tissue water for sodium and chloride are 1.5 to 5 times greater in papillary tissue than in cortex. The extent of the concentration gradient is greatest during antidiuresis at low solute outflow rates, diminished by hydropenic osmotic diuresis, and least during water diuresis. Under all circumstances studied, these ions tend to retain a mutual concentration ratio in tissue fluid similar to that characteristic of extracellular fluid in general, although their absolute concentrations in papillary tissue water (Na 550; Cl 370 mEq. per liter) may reach four times the values simultaneously observed in systemic plasma. By contrast, values for tissue potassium show no such gradient. They are approximately equal in cortex and papilla, decline slightly from cortex to inner medulla, and are unaffected by alterations in urine flow. Values for these three electrolytes in tissue are virtually independent of large changes in their respective concentrations in urine. Urea, however, while having a concentration gradient like that for sodium and chloride, appears to be in equilibrium with plasma urea in cortex and with urine urea in papillary tissue.

The independence of tissue and urine concentrations for sodium, potassium, and chloride suggests that residual urine in the tissue contributes not more than 5% to its volume. Retained blood contributes a similarly small fraction. The constancy of the concentration ratio of sodium and chloride, the absence of a potassium gradient, and geometric considerations suggest that cell volume is diminished and interstitial fluid increased in papillary tissue during antidiuresis and that these changes are reversed in diuresis. In antidiuresis the osmotic pressure of papillary tissue fluid estimated from the presumed osmotic activity of its analyzed constituents is slightly greater than that of simultaneously formed urine. In diuresis, the estimated osmotic pressure of the papillary tissue fluid, though reduced, is far in excess of that of simultaneous urine.

The observations suggest that concentrated urine derives from a dilute percursor fluid contained in tubules, essentially permeable only to water, which conduct it through a medium of high osmotic activity. During this passage water diffuses across the tubular membrane from the precursor to the surrounding medium, the hypertonicity of which is determined primarily by its sodium and chloride content. Dilute urine, on the other hand, is a product of passage of the hypotonic precursor through the tubules (collecting ducts) with essentially no loss of water to the surrounding medium. Antidiuretic hormone appears to control the permeability to water of the membrane of the collecting tubule. In addition, and as a separate function, the hormone appears to effect the marked increase in sodium and chloride content and concentration of the surrounding medium seen in antidiuresis.

Hemoglobin Metabolism in the Premature Infant. JOSEPH DANCIS, JOHN ZABRISKIE,* and M. EARL BALIS,* New York.

N^{15} glycine was administered at birth to two presumably normal premature infants, and the incorporation of the isotope into hemoglobin was measured. The curve of the N^{15} concentration graphed against time revealed four distinct components—a rapid rise in concentration, followed by a more gradual increase, a slow fall, and, finally, an abrupt drop. The life span of the red blood cell and the varying rates of synthesis and degradation of hemoglobin during the first months of life can be readily estimated. The curves obtained contrast strikingly with those in subjects with a stable hemoglobin concentration. There the initial abrupt rise in isotope concentration is followed by a prolonged plateau and then a rapid fall. One of the premature infants developed a more prolonged anemia than the other. The data offer possible explanation for the mechanisms involved.

Discussion

ROBERT A. ALDRICH, Seattle: I want to tell Dr. Dancis how much I appreciate seeing this study initiated. I believe that we pediatricians have not made full use of nonradioactive isotopes such as N^{15} for metabolic studies of infants and children. It was my hope that Dr. Dancis would be able to show us data on the appearance of the isotope in the stool pigments, because with this added information it is easier to interpret the hemoglobin curves just shown. There are two questions I wish to ask: What was the dosage of the N^{15}-labeled glycine that was administered to these premature infants and by what route was it given, and did you divide the dose over a period of hours or days?

CHARLES U. LOWE, Buffalo: Dr. Aldrich has touched on a problem which affects the acceptability of the data presented by these authors. I think it would be important to prove that the N^{15} did not reenter the metabolic pool, because if it did, whereas the points on the curve would be the same, the interpretation would be opposite. In other words, increased synthesis of hemoglobin would produce the curve that the authors have presented, and I wonder whether they are planning to obtain excretion studies in order to confirm their hypothesis.

JOSEPH DANCIS, New York: One gram of N^{15}-labeled glycine per kilogram was administered by mouth.

I strongly suspect there is some reutilization of N^{15}. Other workers have demonstrated that it does

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occur in studies of this type. However, I do not believe that this would affect our interpretation of the data. The half-life of the plasma proteins in these same babies seems to be about six to eight days, indicating that the N^{15} concentration is dropping off rapidly in precursor material and body proteins, thus minimizing the effect of reutilization. The relatively constant total N^{15} in hemoglobin and the falling hemoglobin mass during the first weeks of life also make unlikely the alternative explanation suggested by Dr. Lowe. I think it unwise to attempt to estimate too accurately the life span of the red cell, because of the possibility of reutilization, and therefore we have not attempted to do this.

We are of course curious as to whether the N^{15} is incorporated in bilirubin. We are not sure that we can collect sufficient stool bilirubin during the first week of life to answer the question, but we plan to try.

Electron Microscopy of Experimental Renal Diseases. CAROLYN F. PIEL; LUTHER DONG,* Stanford; JOSEPH A. GOODMAN, and ROGAR MOORE,* Veterans Administration Hospital, Long Beach, Calif.

Electron microscopy reveals that the normal glomerulus is composed of three definitive layers: the inner endothelium, with its attenuated cytoplasm lining the lumen of the capillary; the homogenous basement membrane, and the epithelium, which surfaces the capillary with distinct foot-like processes. The presence of an "inner mesenchymal core" is debated. Alterations in these structures induced by acceptable experimental techniques indicate fundamental differences in degenerative and proliferative processes which are not readily demonstrable by light microscopy.

One hour after the intravenous injection of potent nephrotoxic rabbit serum into rats the electron microscope reveals swelling of the epithelial foot-like processes which proceeds rapidly to complete coalescence, so that the normal spaces between the processes are no longer present, the so-called "smudging of the epithelial layer." This change is associated with subsequent swelling of the basement membrane. These histologic changes are accompanied by the clinical sign of nephrosis. Similar histopathologic alterations are found in biopsied renal tissue from children with nephrosis.

Three hours after the intravenous injection of horse serum into rabbits thinning of the basement membrane was observed, associated on the seventh day with proliferative changes. This proliferation was seen at the same time with light microscopy. Electron microscopy indicates that the proliferation is restricted to the endothelium. Similar observations have been made in children with glomerular nephritis.

These experimental processes not only serve as useful tools for studying comparable clinical diseases but also indicate the nature of glomerular structure and possible alterations under varying influences. In this study no evidence of a mesenchymal stalk has been observed, even after the stimulative influence of heterologous serum.

Electron Microscopic Pathology of the Various Forms of the Nephrotic Syndrome. ROBERT L. VERNIER,* MARILYN G. FARQUHAR,* JOEL G. BRUNSON,* and ROBERT A. GOOD, Minnesota.

The nephrotic syndrome occurs in association with a variety of disease processes and has been observed to follow bee stings, medication with trimethadione (Tridione), and exposure to mercury and other chemical compounds. The majority of cases of the nephrotic syndrome in children, however, are idiopathic, and no associated etiologic factor can be identified. Present clinical and laboratory studies do not afford means of differentiating between the several forms of the nephrotic syndrome listed above.

The renal pathology of the nephrotic syndrome, as studied on postmortem material, has been extremely variable and has been of little value in clarifying concepts concerning the interrelationships of its various forms, its etiology, or its pathogenesis.

During the past two years we have performed renal biopsies on approximately 100 children with chronic renal disease of childhood. All of these renal biopsy specimens have been studied by light microscopy, and approximately 50% have been studied by electron microscopy. In these studies the renal biopsy has been shown to be a reasonably safe and useful procedure for diagnosing and evaluating the progress of chronic renal disease in childhood. Adequate material for thorough morphologic study and pathologic diagnosis is obtained in approximately 75% of the children in whom renal biopsy is attempted.

Application of electron microscopy to the study of renal biopsy specimens in the nephrotic syndrome has revealed a characteristic abnormality involving the epithelial-cell layer of the glomerular capillaries. The present study was undertaken in an attempt to determine whether the renal biopsy of patients with nephrotic syndrome of the several different types might reveal characteristic changes reflecting the basic etiologic process.

Renal biopsy specimens from children with the following forms of nephrotic syndrome were studied by light and electron microscopy: (1) nephrosis following bee sting (2 cases), (2) nephrosis occurring after trimethadione therapy (1 case), (3) nephrosis occurring after exposure to mercury (1 case), (4) nephrotic syndrome

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occurring in association with advanced chronic glomerulonephritis (3 cases), (5) nephrosis occurring in association with polycystic disease of the kidney (1 case), (6) nephrosis occurring as a familial disease (5 cases in two families), (7) nephrosis occurring as idiopathic lipoid nephrosis (14 cases).

The pathologic analysis of the biopsy material in these cases, employing light microscopy, included normal kidney, polycystic disease of the kidney, proliferative nephritis, membranous glomerulonephritis, and subacute and chronic glomerulonephritis.

Electron microscopy, however, revealed a consistent abnormality of the epithelial cells of the glomerular capillary membranes in all these cases. The epithelial foot processes are distorted into a homogeneous essentially uninterrupted mass along the epithelial surface of the basement membrane.

This abnormality has not been observed in diseases of the kidney other than those in which the nephrotic syndrome occurs. We interpret this specific ultramicroscopic morphologic renal abnormality to represent either the basis for, or a consistent reflection of, the essential pathogenic process resulting in the nephrotic syndrome, regardless of its etiologic basis.

The possible mechanism by which the common renal lesion is produced by these diverse etiologic processes will be considered.

Discussion

MAX WACHSTEIN, New York: I should like to ask Dr. Vernier whether the epithelial changes which he described are restricted to cases of the nephrotic syndrome. In other words, do not cases of chronic nephritis also show similar changes?

As a pathologist, I am somewhat perturbed that the mesenchymal cells are again so much questioned. It is very hard to understand how the glomerulus can completely be replaced by connective tissue unless there are somewhere connective tissue cells present to proliferate. You would have to alter basic concepts of pathology very markedly if you would now assume that epithelial cells become histocytes or connective tissue cells.

A far as the staining reactions that were shown here are concerned, I should very much like to ask why in the combined PAS-Hale stains the basement membrane is blue and apparently is not PAS-positive, but if one does not combine the two stains and uses PAS stains only the basic membranes are PAS-positive.

HENRY L. BARNETT, New York: I think that many of us who have been struggling to define and classify these various diseases on the basis of clinical, chemical, and physiologic data find these studies by electron microscopy very exciting.

I am particularly interested in the relationship of the experimental disease to the human disease. I should like to know if Dr. Piel or Dr. Vernier know of any electron microscopists who have studied the disease produced by the "duck-rabbit-duck system." It would appear that this disease, in contrast to the "rat-rabbit-rat disease," has some similarity to but some major differences from nephrosis.

WALTER HEYMANN, Cleveland: I want to ask Dr. Vernier for some information. Mercury nephrosis has been talked about by Fanconi and by English workers of such standard that we do not dare to doubt its existence. Personnaly I do not remember having seen a case of it. I wonder whether you could give us some information regarding the exposure to what kind of mercury and at what age it occurred. I should like to say the same about the "bee-sting" nephrosis. I have read about it, but I have never seen it. I just wonder how one is sure that in summertime, when millions of bees sting thousands of children, some of whom could get nephrosis without having been stung, the disease is due to the sting. I wonder if it has been observed that a second bee sting has elicited a relapse in a child that was in remission from a first attack.

KURT LANGE, New York: I should like to ask Dr. Piel whether or not she has found in her beautiful studies that protein leakage, as such, through the glomeruli independent of any disease causes the same change of the podocytes of the epithelial cells. If this is so, one could possibly interpret the spreading of the podocytes as a "sealing off" process against protein loss.

I would like to ask whether, for example, egg albumin was given to rats, which they are known to excrete very rapidly. Can one note a spreading of the podocytes under such circumstances?

JOHN M. CRAIG, Boston: I think some of Dr. Vernier's photomicrographs show some of the difficulties one has with such renal biopsies. I know that even with wedge biopsies of the cortex we have had difficulty in interpreting the disease process, and it must be even more difficult with needle biopsies.

In regard to the swelling of the podocytes, I think this may be a quite nonspecific process. By use of amino-nucleoside for the induction of nephrosis with Dr. Leon Weiss, of the Department of Anatomy at Harvard, we have been able to demonstrate, even before very marked or easily observable proteinuria, swelling of the podocytes. This system is somewhat more precise to work with, because one does not get the cataclysmic alterations of the glomerulus in the acute stage as you do with the anti-rat-kidney rabbit serum.

WALLACE W. MCCRORY, Philadelphia: I think that these papers, like most in this Society, not only bring new information but pose new puzzles.

I should like to know if Dr. Piel can give us information on the time of the onset of proteinuria in her animals given foreign-protein kidney disease. Dr. Dixon has found in working with foreign-protein experimental disease that proteinuria does not occur with repeated injections of bovine albumin until he can demonstrate localization of the serum albumin by immunologic techniques in the glomerulus. Here, at least, then, is a demonstration of an association between the time of localization of antigen-antibody complex in the glomerular membrane and the onset of proteinuria as evidence of renal damage. Though I am not a pathologist, I should appreciate comment by the authors on the limitations presented to the morphologist in deducing from what is seen how much is known about what a membrane is doing. I find it a little difficult to understand how the epithelial podocytes can be concerned with the process of filtration. What is the functional significance of smudging of the podocytes? Dr. Charlotte Liu, in our laboratory, has been working with tissue-culture preparations of animal and human kidneys. Though we are not clear about the cell types growing in the tissue culture, the suggestions of persons more expert than I is that the cells are probably epithelial. It thus may be of interest that the rat tissue-culture preparations show cytotoxic effects when exposed to nephrotoxic sera. I wonder if this finding bears any relationship to the results presented here. I raise these questions to try and provoke a little more discussion about the significance of the observable pathologic lesions and the as yet unsolved question of the functional alterations responsible for proteinuria.

Carolyn F. Piel, San Francisco: Dr. Wachstein, I am in no position to discuss your question well. However, it seems to me that electron microscopic studies which have been done in in disease states seem to indicate that there is little ground for believing that a mesenchymal core exists. I think that the reconstructions of the glomeruli which were done independently by Hall and Boyer indicate that the glomerular capillary is not in loops but is rather a true vascular network. These reconstructions, the work of Irvine, which we quoted here, and the proliferating cells, surrounded by basement membranes which we see in our rabbits injected with horse serum, lead me to think that the concept of the mesangium needs reconsideration.

The basement membrane was not blue, and if I said this I did not intend to say it. The part of the wall that stains blue is the epithelial surface. The thickened part, which I believe to be the basement membrane and which stains positively with periodic acid-Schiff reagent could be seen most clearly seven days after injection of nephrotoxic serum.

Dr. Barnett, we are extremely eager to work with duck serum, and also with smaller doses of rabbit serum, since it is our belief that one can induce disease in rats with small doses of rabbit serum which is similar to the disease induced with duck serum. These studies are in process.

Dr. Lange, we have not given egg albumen to experimental animals, and this is a very good suggestion. I, too, have wondered which of all these lesions is associated with protein leakage. I do not know the answer to that.

Dr. McCroy, proteinuria in the nephrotoxic rabbit serum disease and in nephrosis, as we see it in childhood, is puzzling to me, and I do not know the answer. We though originally that the situation was clear in the rat disease, because when one begins to see smudging of epithelial cells, which seems to occur first, the rat is anuric and by the time one sees proteinuria the basement membrane has become thickened. As you suggest, it seems more reasonable that the basement membrane should leak protein before the epithelium is involved. I do not know what epithelial reaction represents. Dr. Vernier will comment, I am sure, but maybe it is a reparative situation. I do not think we know.

In these particular animals which received horse serum, we unfortunately only did protein determinations on the urine at the time of killing, and in none of them was there proteinuria. However, proteinuria does occur in association with this type of proliferative disease.

Robert L. Vernier, Minneapolis: Dr. Wachstein's and Dr. Lange's questions regarding the specificity of the abnormality we have described may be answered together. We have studied the ultramicroscopic renal glomerular pathology of a number of cases of acute, subacute, and chronic glomerulonephritis; anaphylactoid purpura, and disseminated lupus erythematosus in addition to the cases of the nephrotic syndrome described in the present paper. The abnormality of the epithelial-cell foot processes which we have discussed has been the principal morphologic alteration in every case of the nephrotic syndrome which we have studied. Similar distortion of the epithelial-cell foot processes has been observed in the other diffuse renal vascular diseases listed above, only in association with severe generalized deformity of the glomerular capillary. In the severely damaged glomerulus, the hyalinized glomerulus of light microscopy, electron microscopy reveals advanced distortion of all three components of the capillary wall, the changes being similar from case to case regardless of the type of renal vascular disease responsible for the damage.

However, very early in the pathogenesis of the nephrotic syndrome, when the renal biopsy specimen appears to be normal by light microscopy, smudging of the epithelial-cell foot processes has

characteristically been observed, whereas the remaining components of the capillary loop are normal.

We have obtained as many as four serial biopsies in the same child over an interval of two years and have observed the clinical picture of pure nephrosis early in the disease to be transformed into chronic glomerulonephritis by clinical, physiologic, and pathologic criteria later in the course of the disease. The early renal biopsies revealed only the epithelial-cell foot-process abnormality by electron microscopy. Later biopsies reveal endothelial proliferation and accumulations of basement-membrane-like material in addition to the epithelial-cell alteration, which persists.

The relationship of the epithelial-cell abnormality to proteinuria per se has not been adequately studied. However, renal biopsy specimens from children with chronic glomerulonephritis in whom proteinuria of 4 to 8 gm. per day is observed have been found to have relatively normal epithelial-cell morphology by electron microscopy. In chronic glomerulonephritis endothelial-cell proliferation and accumulations of basement-membrane-like material within and between the endothelial cells characterizes the early ultramicroscopic pathologic findings.

In response to Dr. Heymann's question regarding the details of the case which we have included as mercury nephrosis, I will relate the circumstances which lead us to use this designation. A 6-month-old infant was found to have the nephrotic syndrome. It was learned that the child's diapers had been soaked in a mercury bichloride solution as therapy for a severe diaper dermatitis. The diapers had been rinsed once and applied as needed for an interval of two weeks, one month before edema was recognized. We did not learn of this circumstance until about three months after the symptomatology of the nephrotic syndrome became apparent. Thus, three months after the onset of the disease repeated studies of the child's urine revealed a significant daily excretion of mercury.

Although the evidence that mercury was an important associated etiologic factor in the nephrotic syndrome in this child is circumstantial, the case was included in order to illustrate the variety of circumstances in which the nephrotic syndrome may occur and to emphasize the uniformity of the ultramicroscopic pathology in the disease regardless of the circumstances of etiology or clinical manifestations.

The relationship between bee stings and the nephrotic syndrome, which was first described by Dr. David Rytand, is not clear. We have studied five children in whom this circumstance preceded the onset of the clinical manifestations of the nephrotic syndrome by a short interval. We believe that the relationship between bee stings and the nephrotic syndrome is more than coincidental, and an investigation designed to clarify the possible mechanisms involved is in progress.

Dr. Craig asked a question about the quality of the tissue obtained by kidney biopsy. It should be remembered that the biopsy specimen is placed in an appropriate fixative within three minutes after its removal from the patient and that minimal postmortem change results. There are certain differences in morphology which are apparent when biopsy material is compared with autopsy material, but we feel that the biopsy tissue is most representative of the true morphology of the organ.

The Relation of Serum Levels of Bilirubin to Respiratory Function of Premature Infants. C. ARDEN MILLER and HENRY H. REED,* Kansas.

Some reports on newborn infants with unexplained hyperbilirubinemia have suggested that anoxia may be related to the abnormally elevated levels of bilirubin. Objective evidence of such a relationship has been lacking. A method of clinically evaluating respiratory function is being used at the University of Kansas Medical Center for early identification of newborn infants with respiratory insufficiency. This method, based on the trend of the respiratory rate in the first hours of life, delineates three groups of infants. All infants with respiratory insufficiency are in Group III. Beginning in August, 1956, determinations of serum bilirubin were done on all prematurely born infants during the first nine days of life. This study reports the levels of indirect bilirubin and relates them to the three respiratory groups.

Three infants were excluded from the study because of evidence of hemolytic disease due to blood-group isoimmunization. The 69 remaining infants each had an average of 4.2 determinations of bilirubin performed during the first nine days of life. Infants in Group III had significantly higher levels of bilirubin than infants in Groups I and II. This difference pertained regardless of birth weight. In fact, infants in respiratory Group III who weighed at birth more than 1750 gm. had significantly higher levels of bilirubin than infants in Groups I and II who weighed less than 1750 gm. at birth.

No infant in Group I or II had a maximum level of bilirubin greater than 13.5 mg. per 100 cc. Fifteen per cent of the infants in Group III had a maximum level of bilirubin greater than 13.5 mg. per 100 cc.

These studies indicate that classification of newborn infants according to the trend of the respiratory rate has predictive value for identifying infants who may develop hyperbilirubinemia not associated with proven blood-group isoimmunization.

* By invitation.

Explanation for the association between early respiratory insufficiency and later hyperbilirubinemia is not clearly evident.

Discussion

DR. BOWEN, Syracuse, N. Y.: I should like to ask the authors one question. It seems to me that the cases that show the highest level of bilirubinemia here are the cases that did the most hyperventilating, and, as we know, in excessive hyperventilation there is a tendency toward dehydration. I wonder if any studies were done to determine the dehydration of these infants that showed the higher levels of bilirubinemia.

SYDNEY S. GELLIS, Boston: This paper, I think, represents well the difficulties to be encountered in explaining individual cases of hyperbilirubinemia and indicates that many possible factors underlie the abnormal increases in bilirubinemia. We certainly see many more cases of hyperbilirubinemia which we can not explain than those which we can. We know that various drugs can be related to it; sepsis, blood incompatibility, et cetera—all these may be factors, and this paper attempts to show, I believe, that anoxia itself may be a factor.

In all the various etiologies the exact pathway for the production of hyperbilirubinemia may not be known. I think it is important, however, for the clinician to be aware of the fact that any underlying pathological state may be responsible for hyperbilirubinemia, such as pneumonitis, congenital heart disease, kidney disease, meningitis, or intercranial hemorrhage, and thus, when one is faced with the patient with hyperbilirubinemia, one should immediately consider the conditions for which one may have therapy, such as neonatal sepsis or meningitis, urinary tract infection, or pneumonitis and institute therapy once these possibilities have been considered and therapy is indicated. Otherwise, there is little one can do, and if the explanation does not lie in these diagnoses, one can then think in terms of congenital heart lesions or intracranial hemorrhage, although we are limited in handling these particular causes.

Perhaps most if not all of these etiologies may work by producing anoxia which affects the enzyme system within the liver. I think the point made about dehydration is a very important one when we have a patient with hyperbilirubinemia which we can not explain. We give subcutaneous fluids in an attempt to see what this will do to the level before considering a replacement transfusion. If the bilirubin will drop below dangerous levels, we are then spared giving such a patient a replacement transfusion, which is dangerous in itself.

One other point should be mentioned. All the levels mentioned in this study were apparently indirect bilirubin. I think one can not avoid determining direct-reacting bilirubin as well, for we have seen a number of cases spared replacement transfusion by the finding that the direct-reacting bilirubin level was rather elevated.

WILLIAM A. SILVERMAN, New York: Would the authors consider the possibility that the association between Grade III respiratory pattern and hyperbilirubinemia might be the result of a dependent relationship of each of these characteristics upon immaturity? I was interested to see that the infants in Grade III exhibited peak values of bilirubin later than would be expected in mature infants. May I ask what the environmental temperatures were? This factor influences the respiratory rate of newborns.

WILLIAM J. WATERS, Syracuse, N. Y.: I think the point of "immaturity" in these cases may be of considerable significance from a number of standpoints in relation to the bilirubinemia. In our determinations of urinary glucuronide excretion in newborn babies, and particularly in the premature infants, the total varied with the urinary output. Dehydration then might be an additional handicap on the conjugation mechanism, which, it appears, is relatively inefficient in this period.

A further possible contributing factor in the elevated bilirubin levels, which I thought Dr. Silverman might comment on, is the question of the relationship of antibiotic therapy. Last year his group reported an increased incidence of brain damage in premature infants on a regimen of penicillin and sulfisoxazole (Gantrisin), and I notice that these infants were given therapy.

Vitamin K has also been implicated in bilirubinemia with brain damage, and along this line Dr. Zinkham has just shown very beautifully its possible hemolytic effect in newborns.

Therefore, it should be emphasized that these and possibly other agents may be competing with bilirubin for conjugation and, if they are better competitors, may then lead to increased bilirubin levels.

All of these factors need evaluation, in addition to the oxygen therapy, in their possible relationship to the increased bilirubin levels that were demonstrated.

PAUL M. TAYLOR, Pittsburgh: I should like to cite our nursery experience and ask a question. We have been studying bilirubin, and we have been impressed in a few cases with the occurrence of rather marked dyspnea in some patients with elevated bilirubin levels. We have been one center, though, that has been very quick to do exchange transfusions on babies with unexplained hyperbilirubinemia. In the past two years we have done about 25 exchange transfusions. In reviewing these, we found that perhaps four or five of these patients had a stormy respiratory course and would fall into Group III of the cases presented. The rest of them are quite all right from the respiratory standpoint, fairly close elevation. We, too, follow

respiratory rates very closely. One factor that came up in our study of hyperbilirubinemia—I saw it mentioned also in a paper submitted by Holman, in Baltimore—is that white babies had a significantly higher peak bilirubin than Negro infants. In our series the peak bilirubin was half again as high in a white as in a Negro baby, a very definitely significant difference. I should like to ask the authors if they divided their patients into Negro and white subgroups in the three respiratory groups.

WALTER R. HEPNER JR., Columbia, Mo.: The original descriptions of neonatal respiratory distress remark on its association with fluid retention at birth. These observations seem worth while to point out, since some comments have been made in the other direction—that of dehydration.

Now, we see an association between respiratory distress and icterus. Some have noted a rather more early and more intense icterus in some infants who have a Cushingoid appearance in the birth room and within the first day or two after birth have pitting edema of the scalp, the chest, the extremities, and the backs of the hands, which are swollen like boxing gloves.

Two questions come to my mind under these circumstances. First, can dehydration be estimated in these babies by a low urine output? Oliguria is not necessarily any indication of the state of hydration. Many infants who are severely edematous put out very little urine during the first two or three days.

Second, one would wonder whether or not the causes of the icterus and the respiratory distress might not be similar to and based on, or related to, inefficiency of edematous organs.

Have the authors attempted to make a correlation between those babies who appear more water-logged who have respiratory distress and icterus? Does this hold?

And then, finally, since these were Group III babies, one would wonder how many of these patients were given oxygen. We understand from Dr. Smull's presentation that oximeter readings were made. Is it possible to draw a correlation between oxygen therapy and icterus? It is well known that higher than usual levels of oxygen in the blood can interfere with some enzyme systems. If correlations can be made between oxygen therapy resulting in very high oximeter readings and the icterus described, the question would be raised whether or not there might be some such relationship between oxygen therapy and icterus in these patients.

GEORGE S. HUSSON, Syracuse, N. Y.: I wonder if the authors have any correlation between arterial saturation and high bilirubin level. Also, did they make hematocrit studies in cases of high bilirubin level?

C. ARDEN MILLER, Kansas City, Kan.: In answer to Dr. Bowen, we have had the impression that the Group III babies have something different about their hydration. This does not appear to be dehydration. We have the subjective impression that they are overhydrated as compared with the Group I and II babies. During the course of this study accurate observations were not done on hydration. We are in the process of doing those studies now.

From Dr. Gellis' comments I did not detect a question, but I did detect the veiled intimation that we did not observe our babies carefully enough to exclude known causes of jaundice. All I can say is that we are cognizant that sepsis, meningitis, et cetera, can cause jaundice, but we believe that those conditions did not pertain to any of the babies in this study. We did determine direct-reacting bilirubin as well as indirect-reacting. I did not report the values for direct-reacting bilirubin here because they did not seem pertinent to the discussion. I want to remind Dr. Gellis and all of you that it was he, and not we, who said that anoxia was an etiologic factor in hyperbilirubinemia. This is a conclusion which we are not yet ready to draw.

Dr. Silverman, I think that it is possible to assume that the hyperbilirubinemia as well as the "Group-III-ness" is due to immaturity. However, I feel that when one says immaturity this really tells very little. We are anxious to find out in what specific respects these infants are "immature."

In regard to Dr. Waters' comments, I do not know what significance penicillin and vitamin K might have had in this study, except that all the babies received the same amount of these agents and there obviously was a difference of bilirubin levels in the Group III babies as compared with those in Groups I and II.

Dr. Taylor, 85% of the premature infants in this study were Negro. In attempting to make any correlation between race and bilirubinemia or respiratory group, we found that the correlations were not significant. This may be a result of the small number of white babies in the study.

Dr. Hepner, we could make no correlation between the levels of bilirubin and whether or not oxygen was given among the Group III infants. We could make no correlations between the level of bilirubin and the oximeter readings. Lack of correlation may be due to small numbers. Hematocrit determinations were not done.

Development of Seizure Patterns in Young Animals: The Significance of Brain Carbonic Anhydrase Activity. J. GORDON MILLICHAP,* Albert Einstein College of Medicine. Introduced by HENRY L. BARNETT.

Experimental seizures were studied in rats and guinea pigs from birth to 35 days of age, and the

* By invitation.

alterations observed in the pattern and threshold of the induced seizures were correlated with the age and maturity of the animal and the development of carbonic anhydrase activity in the brain. Seizures were induced by electroshock stimuli of 50, 100, and 200 ma. applied by corneal electrodes. In young rats aged 20 to 35 days the electroshock seizure was mainly tonic in pattern, whereas the clonic type of seizure predominated in rats aged 10 to 20 days. From birth to 10 days, neither tonic nor clonic seizures could be elicited.

The failure to induce convulsions in the newborn rat was associated with a low level of carbonic anhydrase activity in the brain. Between 10 and 20 days of age, a fourfold increase in enzyme activity occurred concomitantly with the development of a clonic seizure response, and from 25 to 35 days maximal brain enzyme activity was associated with the induction of maximal and mainly tonic seizures. The degree of maturation of the newborn guinea pig was equivalent to that of the 15-day-old rat, with regard both to the type of seizure response and to the level of brain carbonic anhydrase activity.

While the tonic component of the electroshock seizure was abolished by the inhibition of brain carbonic anhydrase with acetazolamide (Diamox) in small dosage, the clonic seizure, which may be induced when the development of brain enzyme is minimal, was controlled only by large doses of enzyme inhibitor. Clinically, it has been observed that in infants and children with generalized myoclonic and grand mal seizures the tonic seizure patterns were controlled with acetazolamide more effectively than the myoclonic jerks.

These observations suggest that brain carbonic anhydrase is of functional significance in the development of susceptibility to maximal tonic convulsions, in which a generalized spread of the seizure discharge is probably involved. The possible role of brain carbonic anhydrase in the development of susceptibility to minor or clonic seizures remains to be clarified.

Discussion

THOMAS H. SHEPARD, Seattle: I should like to ask if there is any correlation between the amount of carbonic anhydrase and the amount of white matter.

In our laboratory we have demonstrated in human material that the larger part of the carbonic anhydrase is located in the white matter rather than in the gray matter.

H. W. BAIRD, Narberth, Pa.: I wonder if the author would comment on the clinical phenomenon which we have observed—I think others have too—that with acetazolamide there may be a good response initially—this is in children with either myoclonic seizures or grand mal seizures—but this good response disappears in time. I wonder if he would speculate on possible causes for this.

SOLOMON A. KAPLAN, Brooklyn: As Dr. Millichap knows, there is direct correlation between the ratio of brain sodium and plasma sodium and the electroshock seizure threshold. As he was presenting his paper I was trying to figure out if the carbonic anhydrase in any way could have affected the transfer of electrolytes across the cell membranes of the brain. This might conceivably provide the link between the activity of carbonic anhydrase and the lowering of the electrolyte shock threshhold. I should like to ask Dr. Millichap if he would like to speculate on what the electrolyte concentration in the brain is and to give us any data if he has them.

WILLIAM BERGSTROM, Syracuse, N. Y.: In red cells, at least, it is very difficult to reduce carbonic anhydrase concentration below about 30% of the normal level. That is, the enzyme, though greatly reduced, is not entirely abolished. I wonder whether this is also true of this brain tissue and whether this might relate to the result.

J. GORDON MILLICHAP, New York: The comment by Dr. Shepard concerned the relatively high concentration of carbonic anhydrase in the white matter of the brain: According to a report by Ashby in 1944, however, in animals, a greater amount of carbonic anhydrase was found in the cortical gray matter than in the underlying white matter. Also, in the precentral gyrus or motor cortex of the human brain the enzyme was found in greatest concentration in the gray matter. In other areas of the human cerebrum the maximum concentration of carbonic anhydrase occurred in the white matter immediately underlying the cortex. Ashby has postulated that the enzyme may be of importance in relation to the nerve fiber and the speed of propagation of the nerve impulse.

With regard to Dr. Baird's question concerning the development of tolerance to the anticonvulsant effect of acetazolamide, we have demonstrated this phenomenon conclusively both in animals and in children with seizures. We have been unable to determine the mechanism of the tolerance, but we have found that it is not associated with a significant decrease in the percentage inhibition of brain carbonic anhydrase.

Dr. Kaplan's question concerned the relation of carbonic anhydrase to the ratio of brain and plasma electrolyte concentrations: In an investigation of the anticonvulsant action of acetazolamide, we found that the inhibition of brain carbonic anhydrase was associated with an increase in the ratio of intracellular to extracellular potassium in the brain and a decrease in total brain sodium. In a recent report, Woodbury and Koch have shown that a decreased intracellular brain sodium concentration induced by acetazolamide is accompanied by a de-

creased radio-Na turnover. It appears that the anticonvulsant effect of acetazolamide is associated with a reduction of the influx of sodium into the brain cell.

Dr. Bergstrom and Dr. Wallace and their colleagues were the first to demonstrate the anticonvulsant effect of acetazolamide clinically. In animal experiments, we have found that brain carbonic anhydrase is inhibited 98% by anticonvulsant doses of the drug.

Problems of Localization of Cellular Change in Renal Tubules in Potassium Deficiency and in Acute Alkalosis. M. A. HOLLIDAY, L. G. WELT,* R. W. WINTERS,* W. HOLLANDER,* W. E. SEGAR,* J. OLIVER,* and M. MACDOWELL, Pittsburgh.

Lesions in renal tubules have been frequently described in chronic potassium deficiency and also in acute metabolic alkalosis in both clinical and experimental states. Opinions have varied as to what part of the tubule is affected. Using the microdissection technique, we have been able definitively to locate the sites of the lesion in these two circumstances. Chronic potassium deficiency is arbitrarily defined as a state of potassium depletion of muscle cells frequently but not always associated with extracellular metabolic alkalosis. The state was produced by providing a diet devoid of potassium to young mature rats for a period of 10 or more days.

From microdissection studies a lesion was observed to occur in some but not all of the proximal convolutions. This consisted of hyperplasia and swelling of the epithelial cells. More extensive changes were noted in the collecting tubules and affected all of them. These consisted of a similar hyperplasia and swelling of the epithelial cells in the first portion of the collecting tubule and, in addition, a marked hyperplasia of intercalated cells. In addition, in the inner zone of the medulla the papillary epithelium was characterized by the development of droplet or granule formation of the cytoplasm. The localization and distribution of these changes were confirmed by dissection of individual nephrons and subsequent histologic study of them. To determine whether the changes observed were dependent on alkalosis or on sodium loading the following experiments were carried out. Each of four groups of animals was fed a basal electrolyte-free diet. The control group received sodium, potassium, and chloride. The other three groups received sodium bicarbonate, sodium chloride, and no electrolyte, respectively. The three experimental groups developed potassium deficiency, but serum bicarbonate was 48, 31, and 25 mEq. per liter, respectively. Despite these differences in sodium loading and alkalosis, lesions were observed in the collecting tubules in all three potassium-deficient groups, and no quantitative difference could be determined among them.

In addition, a diminished ability to concentrate urine maximally was observed in the experimental groups but not in the control groups. The changes in the collecting tubule and the diminished concentrating ability occurred independently of sodium loading or alkalosis.

Preliminary observations in acute alkalosis have cited variations from those described in chronic potassium deficiency. Acute alkalosis was achieved by peritoneal dialysis, with use of a sodium bicarbonate solution. Experiments were terminated three days after dialysis, and kidneys were examined at that time. In the three-day interval potassium deficiency developed from a urinary excretion of potassium in response to the alkalosis. The changes in the renal tubules in the alkalotic group differed from those with chronic potassium deficiency in two important respects. There were no changes in the collecting tubules in the alkalotic group, but the changes in the proximal convolutions in this group were more intense and involved many nephrons rather than a few as was the case in potassium deficiency. The absence of a collecting lesion might be related to a factor of time. It was noted previously that changes in the collecting tubule in chronic potassium deficiency do not appear until after a week. The potassium deficiency in the acute alkalosis experiment was of only three days' duration. The changes in the proximal tubule, however, appear to be a response to an abrupt change, presumably that of alkalosis.

Discussion

ROBERT E. COOKE, Baltimore: I think it might be pointed out that potassium deficiency produced by dietary deficiency and sodium chloride loading with minimal alkalosis will lead to at least a functional proximal tubular lesion with very marked amino-aciduria.

In regard to the alkalosis problem and kidney injury, I think it might be of some interest to the Society to hear of a follow-up of a patient with congenital alkalosis of gastrointestinal origin reported by Dr. Darrow a number of years ago. This child has had alkalosis for 13 years. Renal clearance measurements, renal plasma flow, amino acid excretion, capacity to concentrate, and urinary findings are completely within normal limits, despite this prolonged period of alkalosis.

WALLACE W. MCCRORY, Philadelphia: I should like to ask Dr. Holliday what the differences were in diets other than potassium. Was the protein intake of the rats the same? Dr. Cooke has commented on amino-aciduria or phosphaturia in the animals with the proximal tubular defect.

* By invitation.

ROBERT E. COOKE, Baltimore: The chromatographs of homogenized kidney demonstrated a very marked increase in potassium-deficient animals in the diamino amino acids, comparable to that described by the Cleveland group in muscle. This finding has been described by other authors also, and so there is a distinct biochemical change that can be demonstrated in the kidneys of potassium-deficient animals.

MAX WACHSTEIN, New York: It is very interesting to see that other portions of the nephron, apart from the proximal convoluted tubules, develop significant experimental lesions and that in potassium deficiency such definite abnormalities occur in the collecting tubules. I think it is interesting to note that modern histochemistry has revealed a number of histochemical staining reactions to be positive in the collecting tubules, while they are not in some other portions of the nephron.

Could we hear some more about the granular depositions that Dr. Oliver and his co-workers have described and what the lesions in the collecting tubules more specifically consist of?

DR. MATHEWS, Atlanta: We have been observing a child over the last year and a half who has had an intractable diarrhea with an excessive output of potassium throughout the period. He presented for the first time paralytic spells because of this hypokalemia. We have corrected it to a certain extent, but we have never been able to get his serum level up above approximately 3 mEq. per meter. We evaluated his renal function, and it is evident that there is no intrinsic renal disease, other than that occasioned by his hypokalemia. He has a defect which is manifested by inability to concentrate beyond levels of perhaps 1.010 or beyond approximately that range. This is not responsive to ADH. He is not alkalotic; we can keep his sodium and chloride levels and CO_2 normal by the addition of appropriate electrolytes.

ROBERT J. MCKAY JR., Burlington, Vt.: I should like to ask Dr. Holliday what happens to the appearance of this tubular lesion if the animals are fed potassium after establishing the potassium deficit.

M. A. HOLLIDAY, Pittsburgh: In answer to those questions relating to the proximal tubule (Dr. Cook, Dr. Wachstein), I cited the fact that some changes were observed in the chronic experiment but that only a few nephrons were involved. The point we wished to emphasize was the much greater intensity of the proximal lesion in the acute alkalosis experiments. These same proximal lesions were also intensified in chronic potassium deficiency with loads of phosphate that had no effect on controls, indicating another variable in their development. We have not made any measurement of amino acid or glucose excretion in either group, but such measurements will be done. A few more functioning proximal tubules would result in significant glycosuria.

With respect to the pathology, and the use of other stains, I must defer to Dr. Oliver. The difficulty of correlating histochemical stains for enzymes to quantitative enzymatic measures has been cited by Dr. Lowe. Such observations to date have not been planned.

In answer to Dr. McCrory's question about diet variations, a single electrolyte-deficient diet was used for all studies. Potassium added to the drinking solution prevented any of the abnormalities noted. Pair feeding experiments were not done in the animals used in these experiments. Other data have indicated that it makes little difference.

I think that Dr. Mathews' experience with the child is consistent with that reported in more detail by Drs. Swartz and Hillman, in which this correlation has been observed to a great extent.

In answer to Dr. McKay's question regarding repair, we have some repair studies after one month, and we are able to restore the ability to concentrate urine at least in part and we are able to restore the changes observed in the collecting tubule to virtually a normal state. Scarring and tubular distortion was noted in restitution experiments after long-continued potassium deficiency.

A Histochemical Study of the Rat Nephron in Experimental Nephrosis. HERMAN W. SPATER* and BERTHA A. MASEK,* New York. Introduced by HENRY L. BARNETT.

Kidneys from nephrotic and normal animals were compared by histochemical methods. Four hydrolytic enzymes were studied as well as mucopolysaccharides by the periodic acid-Schiff (PAS) reaction; routine hematoxylin and eosin was also used. Mild nephrosis was produced in a small series of young Sprague-Dawley rats by injection with rabbit serum containing anti-rat-kidney antibodies. Animals were killed 72 hours after injection, by which time nephrosis was evidenced by a ten- to twenty-fivefold increase in proteinuria. Adenosinetriphosphatase and nonspecific esterase activities were not different between the two groups, while PAS and hematoxylin-and-eosin preparations showed only minimal glomerular alterations. Striking changes were noted in alkaline phosphatase activity, which was markedly decreased in the proximal convoluted tubules of the outer two-thirds of the renal cortex, whereas it remained normally active in the proximal tubules of the juxtamedullary area. Equally striking was the virtual absence of acid phosphatase activity in the proximal convoluted tubules of the nephrotic kidney, whereas normal kidney showed abundant acid phosphatase activity discretely localized to cytoplasmic droplets within the proximal tubular cells.

* By invitation.

This study reveals that two enzymatic defects in the nephrotic kidney tubule appear to precede visible alterations seen in routine histological preparations.

Discussion

CAROLYN F. PIEL, San Francisco: I should like to ask one question. Have you given rat serum to increase the protein excretion in a normal rat and observed the same type of droplets? I gather that rabbit serum given to rats produces no alterations.

WALTER HEYMANN, Cleveland: I should like to underline the importance of these studies by pointing out that years ago when we studied the metabolism of the renal cortex we found an impressive metabolic lesion. Glucose and fructose failed to increase the oxygen uptake of nephrotic renal cortices in Warburg measurements. I could very well see that the histochemical changes that Dr. Spater has found reflecting alterations of phosphatase activities could be the cause of the described metabolic lesion.

We found it to be present within a day after the injection of nephrotoxic serum and found it to be present as long as the disease continued, for months and months.

If Dr. Spater is in need of animals with long-lasting, 11- and 12-month-old, disease, I will be only too glad to send him some animals.

CHARLES U. LOWE, Buffalo: I move to comment on this paper not because I know very much about this lesion in the kidney but rather because of the histochemical implications of this presentation. I assume that in the phosphatase stains the authors used Gomori's technique or some modification of it. Both Gomori and Glick, in separate communications, have indicated the hazards of this technique. In working with liver, we have attempted, whenever we used histochemical methods to identify enzymes, to always supplement them with straightforward chemical determinations of the enzyme In liver homogenates, at least with respect to alkaline phosphatase, we have been appalled by the lack of correlation between straightforward enzymatic determinations and the histochemical determinations.

I should like to suggest that histochemical techniques are good for localization but that perhaps one should use caution in judging how much or how little of an enzyme is present unless this can be verified by more ordinary chemical methods.

ROBERT L. VERNIER, Minneapolis: The authors have indicated that they plan to correlate these very nice histochemical data with electron microscopy of the renal tubule. I feel that this approach is likely to be very informative. For example, preliminary electron microscopic studies of the tubules in renal biopsy specimens from children ill with the nephrotic syndrome suggest that some of the hyalin and fatty droplets seen in this disease may be altered mitochondria. Our observations support the concept that tubular mitochondria take up protein and lipid materials and become swollen. Severely swollen mitochondria devoid of the usual internal structural pattern of mitochondria thus resemble the protein and fatty droplets described as a characteristic pathologic feature of the nephrotic syndrome.

ROBERT J. SLATER, Toronto: In hearing these presentations this morning, which have taken us down to a very deep look at the structural changes that occur when the kidney undergoes certain types of damage, I keep coming back in my mind to the fundamental question which each of the persons who has presented a paper is getting at, and that is the pathogenesis of the disease under study. And of course this brings up, as Dr. Barnett so clearly pointed out, the specificity of the lesions being produced. The experimental models that have been used are largely based on antibody-antigen reactions, because that seems to be most closely correlated to what occurs in the human. I should like to ask Dr. Spater if similar types of studies are being carried out now with, for instance, the type of experiment that Ellis carried out a few years ago using saccharated iron oxide, in which he produced a pure nephrotic syndrome in animals. This apparently had nothing to do with an antibody-antigen reaction. Likewise, I wonder if anoxia of the kidney will produce these enzymatic changes in the tubules, or straight injection of toxin into the kidney, again not having anything to do with an antibody-antigen reaction. This all bears on the specificity of the type of damage which will produce these various changes.

MAX WACHSTEIN, New York: I should just like to comment on one particular point in this presentation, and that concerns the appearance of an abnormal phosphatase reaction. If Dr. Spater's work is confirmed, I believe it will be the first instance where definite localized decrease of phosphatase can be demonstrated in renal cells without significant associated changes in routinely stained sections.

The first suggestion that this might happen was presented by Wilmer, in 1941, when he ligated the ureters of rabbits and showed very remarkable decrease in alkaline phosphatase in the tubular cells. He came to the conclusion that under those circumstances, indeed, phosphatase was diminished before histological changes could be seen. When these experiments were repeated very carefully, it was noticed that diminution of phosphatase was associated with cellular changes that could be observed in routinely stained sections.

If one ligates the renal artery in the rat, which of course produces extensive renal necrosis, one can definitely see that phosphatase is still present in the necrotic cells for some time. In experimental rabbit

nephritis phosphatase disappears dramatically, but this diminution does not precede other changes. So, if this difference exists in the rat in this experimental condition I think that that would be a very significant new experimental fact.

HERMAN W. SPATER, New York: In answer to Dr. Piel, we have not used rat serum; however, I think it is an excellent suggestion.

I should like to thank Dr. Heymann for his comments and for his offer of his experimental animals to continue this line of investigation.

As to the third question, the Gomori stain was used. The hazards of these methods are well known, and we are quite aware of them. I will say, however, that work done by Dr. Alex Novikoff, at the University of Vermont, on liver homogenates and various subfractions of the homogenate in which the phosphatases were carefully analyzed show that recovery is good and consistent in the various fractions. These results seem to differ from the variability obtained in your studies.

In answer to Dr. Vernier's question, it seems that the mitochondria are not distributed like the other droplets present, and we also know from centrifugation studies that the rich acid phosphatase droplets are not mitochondria, since this enzyme is not prevalent in mitochondria. Of course, some of the droplets certainly may be swollen mitrochondria that have adsorbed protein, but I think there are others present alongside that could easily be mistaken for those structures.

In reference to Dr. Slater's question about the work that is being done in this field with nephrosis induced by other methods than the antigen-antibody techniques, I can only refer to the vast literature so beautifully gathered and discussed by Dr. Wachstein in a paper on experimental nephrosis in *The Journal of Histochemistry* about two years ago.

In answer to Dr. Wachstein, I can say that several months ago three of our medical students removed some dog kidneys several hours after the ligation of the ureters and found diminution of alkaline phosphatase in the proximal tubules very similar to what was shown here.

The Biochemical Pathogenesis of Galactosemia.

JAMES B. SIDBURY JR.,* Johns Hopkins. Introduced by VICTOR A. NAJJAR.

Dietary galactose is inimical to patients with galactosemia. Recently it has been shown in both liver and red cells that the defect of these patients is a deficiency of the enzyme responsible for the exchange of galactose-1-phosphate (Gal-1-P) for glucose-1-phosphate (G-1-P) in uridine diphosphate glucose (UDPG). Earlier it had been shown that Gal-1-P accumulates in the red cells of these patients when galactose is present in their diet; thus the red cells manifest the defect which is present in all the cells of the body.

The knowledge of the specific enzymatic defect has not clarified the biochemical pathogenesis of the symptoms found, since withdrawal of galactose relieves the symptoms. It has been shown that Gal-1-P reacts with phosphoglucomutase at 1/400 the rate of G-1-P. Phosphoglucomutase has been shown to transfer the phosphate of the enzyme to either the 6 or the 1 position of glucose phosphate, resulting in the formation of glucose-1,6-diphosphate (G-1,6-P) and dephosphoenzyme. Dephosphoenzyme and G-1,6-P react to form glucose monophosphate (G-1-P or G-6-P) and regenerated phosphoenzyme. Gal-1-P must react with phosphoglucomutase by the same mechanism, but doing so at a much slower rate would cause it to be a competitive inhibitor. We have shown marked inhibition of the phosphoglucomutase reaction with both galactose monophosphates. The Gal-1-P inhibition is very rapid. In the absence of G-1,6-P, maximal inhibition (80%) was attained within five minutes with 0.1 millimoles of Gal-1-P and within 30 minutes with 1.3×10^{-6} millimoles. Gal-6-P (2 millimoles) gave maximal inhibition in 30 minutes, and with use of 1.3×10^{-6} millimoles maximal inhibition was not approached in one hour. The rate of inhibition with Gal-6-P is approximately 1/20 that with Gal-1-P, which is in harmony with the kinetic studies of the two steps of the phosphoglucomutase reaction. 1.9×10^{-4} millimoles of enzyme was used in these assays. The results illustrate that concentrations of Gal-1-P approaching the concentration of the enzyme are inhibitory and hence accumulation of Gal-1-P of the order demonstrated in the cells of galactosemia patients with galactose in their diets is commensurate with marked inhibition of phosphoglucomutase in the cells of these patients.

Phosphoglucomutase participates in several essential metabolic reactions, e. g., in the formation and utilization of glycogen, mucopolysaccharide synthesis, and nucleic acid synthesis and, consequently, protein synthesis. Inhibition of the above reactions through inhibition of phosphoglucomutase would explain the symptoms of patients with galactosemia.

Discussion

ANDREW SASS-KORTSAK, Toronto: The question I should like to ask is somewhat off the subject of this beautiful paper. I should like to draw attention to another very interesting phenomenon in connection with the metabolism of galactose. It has been known for some time that galactose tolerance can be improved and the ability to metabolize galactose enhanced in a normal person by the intravenous or oral administration of glucose before the test dose of galactose is given. The explanation for

* By invitation.

this phenomenon is not known. I wonder whether the authors would care to comment on this problem.

RUTH GROSS, San Francisco: I should like to ask Dr. Sidbury if he would be willing to speculate for us as to what he thinks happens when these children grow up and do so much better clinically in the face of their enzymatic defect.

JAMES B. SIDBURY JR., Baltimore: Regarding the observation in your patient that glucose facilitated the utilization of galactose, I know of no proven explanation. This observation has also been made in animals and in vitro.

The adults with galactosemia retain the defect in their nucleotide transferase; however, Isselbacker recently suggested that they may develop an alternate pathway for the utilization of galactose. Recently Dr. Zinkham has obtained evidence on an 11-year-old child with galactosemia suggesting the same; this is only preliminary.

The Influence of Maternal Iron Deficiency on the Newborn. THOMAS R. C. SISSON* and CURTIS J. LUND,* Rochester. Introduced by GERALD MILLER.

An investigation to determine the influence of maternal iron deficiency on the newborn infant was carried out with the use of plasma volume determinations (T-1824 [Evans blue] dye dilution technique) and measurement of serum iron and iron-binding capacity, hemoglobin concentration, hematocrit value, red cell indices, reticulocyte count, serum bilirubin, and total serum protein.

Eighteen anemic women, eighteen nonanemic women, and their babies were selected for comparison. The infants were studied on the fourth or fifth day of life, when shifts of the plasma compartment had stabilized. The serum iron concentrations at this time had returned to stable levels from those of the first day following birth. The mothers were studied repeatedly during pregnancy, and the diagnosis of iron-deficiency anemia was made from values established during the last six weeks of gestation.

In pregnant women and newborn infants measurement of red cell volume and circulating hemoglobin mass determine a state of anemia more accurately than the simple measurement of hemoglobin concentration and hematocrit value. As an example, the gravid women with hypovolemia will be anemic in nearly all cases, though the hemoglobin concentration and hematocrit will be normal or nearly so.

In any study of the blood volume of the newborn infant it is necessary to consider the so-called "placental transfusion" which is said to occur as the result of delayed clamping of the cord. Such a volume of blood, estimated by some observations to exceed 60 ml., would add considerably to the iron reserve of the newly born infant. We have determined the blood volumes of 30 infants with and without the influence of gravity on umbilical blood flow. Neither immediate nor delayed clamping with the infant held below the level of the placenta could be shown to influence the blood or red cell volumes significantly by the third day of life. For this reason we believe that neonatal values are generally unrelated to "placental transfusion" unless the most artificial circumstances and maneuvering of the infant and placenta surround the technique.

The babies born of anemic mothers had a mean circulating hemoglobin mass of 14.0 gm. per kilogram. The babies born of nonanemic mothers had a mass of 16.5 gm. per kilogram. The difference of 2.5 gm. per kilogram is more striking when it is considered that this represents 15% to 20% of the iron available for hemoglobin synthesis in succeeding weeks of life. The mean red cell volume of the infants of the anemic group of mothers was 35.0 ml. per kilogram, and that of the infants of the nonanemic group was 40.0 ml. per kilogram. The red cell volume of the infants of anemic mothers is 13% lower than that of infants on nonanemic mothers.

The individual data for each mother plotted against data for her infant demonstrate that, in general, the lower the red cell volume and hemoglobin mass in the mother the lower these values tend to be in her baby.

It appears that the serum iron of the newborn infant reflects the maternal iron supply but is not directly related to the maternal serum iron values. The 18 babies of anemic mothers had an average of 78 μg. per 100 cc. of serum iron, and the babies of nonanemic mothers had an average 111 μg. per 100 cc. This difference of 40% is significantly greater than the range of values found in either group of infants.

It is rare for a nonanemic mother to give birth to an infant with evidence of iron-deficiency anemia, but our data strongly suggest that infants of anemic mothers will frequently share the iron-deficiency anemia. Treatment of the mother with iron, if effective, tends to relieve the infant of this common deficiency. It may be concluded that the more severe the anemia in the mother the more this will be reflected in the infant at birth. Such a profound effect on the newborn may be expected to influence the production of iron-deficiency anemia in later infancy.

Discussion

ARTHUR E. MCELFRESH, Narberth, Pa.: I wonder, for my own benefit, if you would tell me what the variations in the infant's plasma and blood volume were from one infant to another, related to either kilogram or body surface.

I should like to point out that the data, and, of course, I can not make out the figures exactly, on

* By invitation.

the hemoglobin mass increment following cord stripping, would lead me to believe that 2 or 3 gm. per kilogram is given; this would result in a dose for a 3000 gm. child of about 30 mg. of iron and would be of substantial benefit to this child if true.

The last question, the thing that bothers me most is that the hemoglobin and hematocrit level are equal in both normo- and hypovolemic infants. We must then presume that the plasma volume is much less in these infants given the fact that the hemoglobin mass is less. I wonder if the authors have any explanation as to why they think this occurs.

WALTER R. HEPNER JR., Columbia, Mo.: Some years ago were were interested in placental transfusion of the newborn infant, and the reflection that this transfusion might have in the immediate neonatal course and the later course of the baby. We set out not only to determine what happened in the baby but what had happened in the placenta. We began by assuming that by arbitrary early ligation or late ligation of the umbilical cord that we would be able to control the volume of the placental transfusion. Hematocrit values at four-hour intervals in the newborn infants, in conjunction with plasma protein determinations, disabused us of this thought. Where these babies had a very great hematocrit rise, almost invariably a considerable plasma protein rise also occurred. This association seemed to be convincing evidence of hemoconcentration following a transfusion.

The disabusing of our notion was that some babies had rises in hematocrit and plasma protein values even though immediate attempts had been made to ligate the umbilical cord. Conversely, in some infants in whom we thought we had assured effective emptying of the placenta, we failed. We set about to find out why. We found that there were several factors which could dominate as strongly or more so than cord ligation and were most difficult to control.

One of the important factors seemed to be general anesthesia and whether this anesthesia was deep or shallow. Another factor that seemed to be of considerable importance, and indeed not infrequently overrode our attempts at control by early or late ligation of the cord, was whether or not the baby had cried vigorously before the cord was ligated. If he cried before an attempt to ligate the cord early was made, he nevertheless received a large placental transfusion. On the other hand, even though we might have waited for as long as three, four, or five minutes, if crying had not occurred placental emptying was inefficient. We measured the amount of blood in these placentas and thereby knew our degree of success or failure in emptying the placenta. Inverse correlation between the volume of blood remaining in the placenta, expressed as a percentage of the infant's body weight, and the evidences of hemoconcentration was excellent.

I wonder if this control is not necessary before any assumptions can be made about how much placental transfusion actually was given to the baby?

I should like to remark on the influence of gravity on placental emptying. I have been confused for some time about the interpretation of remarks about the level of the placenta in relationship to the baby and whether the baby gets the blood or not. Most of us who have worked in this area have known for some time that if the clamp of ligature slips from the cord when it is ligated early sometimes blood from the umbilical vein will spurt as far as 6 or 8 ft. I wonder if in the presence of pressures like this gravity is a factor at all.

The last question I should like to ask is this: Although we can see very nicely here that there is a definite correlation between the serum iron levels of mother and baby, before we would be bold enough to be sure that there is an iron-deficiency anemia would we not wish to know something about iron-binding capacity on follow-up of these babies? Do the iron-binding capacities of these babies move in the opposite direction from the serum iron levels as time passes, thus confirming an iron-deficiency anemia?

CALVIN W. WOODRUFF, Nashville: I should like to point out that, so far as I am aware, this is the first bit of evidence concerning the influence of maternal iron stores on the development of anemia in infants that has come since Dr. Straus' work over 20 years ago. Maternal iron deficiency is probably an important factor in the later development of hypochromic anemia. Those of us who have been concerned about the variation in hemoglobin concentration at birth and its possible interpretation now have reason to need the further and better studies Dr. Sisson has made with his estimations of the circulating hemoglobin mass, which is not necessarily parallel with the hemoglobin concentration at birth.

DENMAN HAMMOND, San Francisco: I think there is a lot of evidence in research in this area, and I hope data of this sort will help us resolve some of the nuclear points. I should like to suggest that in order to determine the influence of maternal iron stores on the subsequent hemoglobin behavior of the infant one would have to restrict his study to nonanemic mothers and that the studies of the maternal iron stores in nonanemic mothers might then show some difference in the behavior of the infant subsequently if the maternal iron stores were found to be different in nonanemic mothers. I wonder if in a nonanemic group Dr. Sisson has any such correlation.

Another question I should like to ask is whether the infants that had cords clamped by various

methods were followed for long enough periods to detect, although on the fourth day there do not seem to be great differences in their circulating blood volume and hematocrit level, whether or not they might have received some iron for their stores in different amounts, depending upon different methods of handling the cord.

SYDNEY S. GELLIS, Boston: I just wonder what the authors' final conclusions will be concerning the handling of the cord and the placenta in newborns, at least at their present stage of research. We found that in a number of babies whose placentas were being held very high above the baby for placental transfusion blood went from the baby to the placenta instead.

THOMAS R. C. SISSON, Rochester, N. Y.: At the moment we do have considerable data which we did not present, covering effects on more than 200 infants.

Dr. McElfresh, the variation in blood volume from one infant to another was definitely seen. Infants of anemic mothers had lower plasma volumes, and the blood volumes were also somewhat lower. I wish to repeat again that the factor of hypovolemia, which was not seen in every case of the mothers we selected, but in several of them, did affect the values for the infants that we presented.

The range of values found for plasma volumes, and from them the calculations one makes to arrive at blood and red cell volumes and so on, is very great in the newborn period. The standard deviations of ±28 with a blood volume of 104 ml. per kilogram for the normal newborn infant is, as you can see, very wide. This range narrows, naturally, during the first six weeks of life, but still through infancy, as long as we have been able to follow our considerable number of infants, there is a wide range. No one is very much like another.

Dr. Fashena did some work a few years ago using $I^{\alpha\alpha}$, which she reported before this Society but has not published, giving figures similar to ours in the newborn infant.

As to the cord-stripping, I could talk all day. We do feel that the magnitude of the blood iron that it has been possible to give these infants by stripping the cord was perhaps minimal with the method we used. Ours was to control the stripping to a certain length of cord, a minimum of 45 cm., and to strip the cord five times. We did not attempt to drain the placenta as was done by Duckman—I refer to the method of hoisting the delivered placenta. The blood can certainly drain back into the placenta from the infant.

The increment of iron that may be added by stripping the cord was at least in the order of 30 mg. of iron, which may be essential.

The lack of correlation between hemoglobin and hematocrit value and hemoglobin mass and red cell volume occurs, we are sure, because there is a considerable difference between total quantity and concentration. On the one hand, you are simply measuring the concentration in the blood, and, on the other, with the known limitations in any method of measuring plasma volume, we arrive at a figure which rightly or wrongly we feel is the total quantity.

Dr. Hepner, I am very interested to learn of your feelings about the placental transfusion. This involves the great deal of work of S. R. M. Reynolds, and the whole problem of controlling the factors surrounding so-called placental transfusion involves the hemodynamics of umbilical circulation at birth and for a few minutes afterwards. It is terribly complicated. To control this by measuring the placental blood volume, we feel, is somewhat misleading, because a baby of thus and so size does not necessarily have a placenta equivalent in size to that of a similar infant. Therefore the volumes will be different.

The immediate clamping of the uterus onto the placenta is maintained from the moment of birth, and the effect is not principally that of squeezing the placenta like a sponge, but rather its effect and purpose is to separate the placenta. The squeezing effect is one that has been overrated in the past, I think.

The effect of gravity is an artificial one, and most of the literature refers simply to delayed ligation of the cord, and, as we think we have shown, simple delayed ligation does not affect placental transfusion.

You can produce a transfusion with artificial measures, and I believe that Reynolds feels very strongly that gravity is of major importance, and of course Duckman's work has also shown that it can be.

The iron-binding capacities were not reported because in the newborn period there very often is not any latent capacity.

The average latent iron-binding capacity for the newborn infant is something on the order of 50μg. per 100 cc.; in some infants it is 100μg., and the following day it may be zero. It is extremely difficult to reproduce, and it is considered normal if there is no latent iron-binding capacity at all.

Dr. Hammond, the restriction of the studies to nonanemic mothers is something that we wish we could do. Unfortunately, our mothers come to a clinic, and they are almost all anemic. We had to really hunt around to find nonanemic mothers and our professor of obstetrics was kind enough to donate some of his private patients. We do feel that the economic state and dietary history during pregnancy are important in producing or preventing iron deficiency in the infant. A correlation does, I think, exist.

No, we do not calculate different amounts of iron by the various methods except where we stripped

the cord, and Dr. McElfresh's calculation of 30 mg. of elemental iron is, I think, very close to it.

Dr. Woodruff, I think that the hemoglobin mass circulating is certainly of far more moment in assessing these infants and, not only that, in assessing a subsequent development of hypochromic anemia than any number of hemoglobin concentration measurements. I certainly do not mean to say that the hemoglobin concentration is valueless. It is of considerable value, and unless the laboratory is prepared to do blood volume determinations we must rely on it. However, I should like to emphasize that there is a large number of people of either pediatric or adult age who do not fall in the isovolemic group; 33% of our pregnant women and 25% of our newborn infants are not isovolemic but are either hyper- or hypovolemic.

Our final conclusion, Dr. Gellis, concerning the placental transfusion, is that it needs considerably more study before we advocate any one procedure, and I do not feel that we have reached the final answer.

The Regulation of the Level of Alveolar Ventilation. MILDRED STAHLMAN, Tennessee. Introduced by AMOS CHRISTIE.

It has been generally accepted that arterial CO_2 tension is one of the factors regulating the level of alveolar ventilation, mediated through a chemoreceptor in the medulla, and that this center has a threshold for arterial CO_2 tension, below which CO_2 has no effect.

With use of techniques previously described before this Society, the arterial CO_2 tension and alveolar ventilation were measured in 37 normal newborn infants. These values showed no correlation. However, when CO_2 output was plotted against alveolar ventilation, the arterial CO_2 tension fell on a series of straight lines arising from the origin, being higher when the CO_2 output to alveolar ventilation ratio was high and lower when it was low. When this ratio was plotted against arterial CO_2 tension a straight-line correlation resulted. In addition to the 37 newborn infants, these data include determinations on 2 normal infants studied 7 and 27 times, respectively, during their first five weeks of life and four adults studied at rest and after exercise on a tread-mill and similarly after they had received progesterone and salicylates as respiratory stimulants and then exercised. Also included are data from two circumstances where CO_2 was not the only stimulant to the respiration, namely, three adults studied during voluntary hyperventilation and 10 studies of various levels of exercise during 10% O_2 inhalation. No relationships could be shown for any of these persons or circumstances between alveolar CO_2 tension and alveolar ventilation, except as alveolar tension was regulated through the CO_2 output to alveolar ventilation ratio.

Newborn infants who were subjected to inhalation of 2% and 4% CO_2 for 10 minutes to 1 hour showed erratic correlation between alveolar ventilation response and alveolar CO_2 tension. However, when CO_2 output to alveolar ventilation ratios are plotted against the alveolar CO_2 tension minus the inspired CO_2 tension, these also fall on the same straight line.

When arterial or alveolar CO_2 tensions were followed on subjects before, during, and after cessation of 4% CO_2 inhalation, it was found that arterial CO_2 tension failed to change or rose slightly during CO_2 inhalation and that alveolar ventilation increased, but that after CO_2 inhalation was stopped CO_2 output remained higher than normal for some time, as did alveolar ventilation but that alveolar CO_2 tension fell considerably below control levels. The same series of events follows the cessation of exercise.

If one determines the mixed venous CO_2 tension during circumstances of increased CO_2 production which do not change the cardiac output, a good correlation can be shown between mixed venous CO_2 tension and the level of alveolar ventilation. If, however, cardiac output increases to the same extent that CO_2 production increases, or if arterial CO_2 tension is lowered as the result of recent hyperventilation, the mixed venous CO_2 tension may remain unchanged.

These relationships between cardiac output venous and arterial CO_2 tensions, and alveolar ventilation lead to a hypothesis that the site of the chemoreceptor may be at the tissue level. Man does not live in a steady state but has minute-to-minute changes in his metabolic CO_2 production. The relative efficiency with which his cardiovascular and respiratory mechanisms of tissue CO_2 removal respond to these changes in production determine the levels of his arterial and mixed venous CO_2 tensions, rather than either of these being the primary stimulants in themselves.

Discussion

SYDNEY SEGAL, Vancouver, B. C.: I should like to congratulate Dr. Stahlman on her courage and initiative in coming up with this new concept after all of the stereotyped explanations we tend to hear.

I have two questions. Many who work in the field of respiration in newborn infants have been intrigued for some time by the manner in which Dr. Stahlman has been able to collect both gas and blood samples without, apparently, disturbing the infant, and I wonder whether Dr. Stahlman is willing to divulge her secret.

The second question is of the nature of the control of respiration in the adult. This is a most contentious subject, as everybody knows, and yet while we are trying to explain an even greater

unknown, that is, the picture in the newborn, we tend still to use these concepts of the adult, which have not been agreed upon, and even proceed to extend these data to explain what we think are differences between the adult and the newborn. I wonder, since she has studied with these methods both the adult and the newborn, whether Dr. Stahlman is willing at this time to say anything about these differences, if they exist, and what she thinks about the probable mechanisms.

JOHN J. OSBORN, San Francisco: I find this a fascinating paper, and it certainly is beautifully worked out, but I find I can not agree with Dr. Stahlman on the conclusions. I have gone over this abstract over and over again, and I still come up with different conclusions.

Going back to one of the first things in the abstract, which also was in the paper, the quotation, "If arterial CO_2 tension on the distal side of the pulmonary capillary bed regulated the level of alveolar ventilation, there should be a good correlation with the level of arterial CO_2 tension and alveolar ventilatory response."

Well, this is what I do not follow, because it seems to me that here we are dealing with rather a typical feed-back control system with a thermostat somewhere in the respiratory center and CO_2 in perhaps the venous or arterial side.

When I used to live in the cold and foggy icy East, I moved a great many times from house to house. I remember that I lived in one house where the thermostat was inside the house. Whenever it got cold outside, inside it got hot. This was an example of excess feed-back. I lived in another house later where every time it got cold outside it got cold inside. This was an example of insufficient feed-back. I am very happy to say that finally I lived in a house where whatever the weather was outside it stayed at 70 degrees inside. Here there was no correlation between the level of temperature outside and the level of temperature inside because we had a properly operating, balanced feed-back.

Now, I have studied about 50 children and adults, measuring their alveolar CO_2 at rest and during exercise, and I have been fascinated by how remarkably constant they maintain their alveolar CO_2 whether they rest or exercise, and this seemed to me very good evidence that the thing which the thermostat is measuring and controlling with a very good and balanced control system is the arterial pCO_2, which is reflected as the alveolar pCO_2 where we measure it.

GEORGE S. HUSSON, Syracuse, N. Y.: I, too, have to echo Dr. Osborn's remarks. In a study several years ago, in a series of 50 cyanotic patients with congenital heart disease, we, too, found that the regulating factor was the arterial pCO_2 rather than the mentioned venous or tissue value.

I have two questions that I would like to ask Dr. Stahlman. In these patients in which we had a direct right-to-left shunt through the arterial system we found that the mechanism was that of slight acidosis. I should like to ask if she has measured pH's. In addition, were these babies in what we would consider a steady state as reflected by the respiratory quotient?

NED W. SMULL, Kansas City, Mo.: I should like to ask Dr. Stahlman to clarify her remark about the lack of uniform response to infants breathing 2% to 5% CO_2.

It has been my experience that there is a very uniform response in respirations of infants breathing 5% CO_2, particularly in the early stages of life.

ROBERT E. COOKE, Baltimore: I should like to ask whether or not these studies were done with large doses of acetazolamide. In the dog, at least, one can dissociate the pCO_2 of arterial blood from the alveolar CO_2 tension in this way.

MILDRED STAHLMAN, Nashville: This is something that I have spent a great deal of time thinking about, and it is very hard to present all of your concepts in a small 10-minute presentation with complete discussion of all the ideas.

As to Dr. Segal's question about how we manage to get both gas samples and blood from newborn infants—the only thing I can tell him is that we feed them and diaper them and rock them, and once they are asleep one can do almost anything you want to them. The techniques which are used for the collection of blood or alveolar gas have all been presented here previously.

His question about differences between adults and newborns: I am certainly convinced that the basic mechanisms for the stimulation of the ventilation, as far as CO_2 is concerned, are the same for adults and newborn infants. However, whether or not there is such a thing as sensitivity wherever the chemoreceptor is, whether this sensitivity is set at a different level in a newborn infant and what the regulating mechanisms of this level of sensitivity are, I am certainly not prepared to say at this time.

Dr. Osborn, I certainly agree with you that this is a feed-back system and that was one of the things which I really tried to express. My concept is that the removal of tissue CO_2 is the necessary part of the homeostatic process and if this removal is not accomplished by the cardiovascular system then it must be done by the excess respiratory effort of the infant, and this excess respiratory effort lowers the arterial and the alveolar pCO_2, increasing the tissue to blood gradient.

If this tissue removal by the cardiovascular system by adequate blood flow is supernormal, the reverse occurs, so that hypoventilation is the

result and the arterial pCO_2 rises. This therefore creates a smaller gradient between the arterial blood and the tissue tension, which allows for reaccumulation of the CO_2 which has been removed from the tissues in excess.

I am not sure that I can answer all of Dr. Osborn's questions with that statement. I should like to discuss this with him later.

Dr. Husson, we have not studied any persons with cardiovascular disease. However, I am reasonably sure that many of these infants do have open fetal shunts at the time at which they are studied. We have not demonstrated any acidosis. We do pH studies on them, and these babies are not in any state of metabolic acidosis. Their respiratory quotients are normal, and the only way that I can convince you of the fact that they are in a steady state is that on the slide which showed CO_2 output divided by alveolar ventilation this was gas measurement and on the other axis it was arterial pCO_2, which was a blood measurement, and these two correlated, as I tried to point out. This is an internal check on steady state and one's ability to measure these values separately.

Dr. Smull, these infants breathed CO_2 at various times during their first five weeks of life. We did not study any infants, I think, under 24 hours of age. However, the problem in CO_2 ventilation measurements in small infants is one of measuring alveolar ventilation and not total ventilatory response. The dead space to tidal volume ratios may change tremendously from one infant to another from one hour to another, and certainly from one day or one week to another. Unless one is measuring alveolar ventilation one can not make any statement about response to any given ventilatory stimulus.

Dr. Cooke, we have not studied any persons or animals with acetazolamide up to date.

The Use of the Pump Oxygenator to Sustain Life During Neonatal Asphyxia of Lambs. H. S. HARNED JR.,* M. A. TANDYSH*, MARY MCGARRY,* J. KEEVE,* and B. KUSSEROW,* Yale. Introduced by LYTT I. GARDNER.

Recent studies of neonatal asphyxia in lambs have revealed a pattern of biochemical changes and circulatory degeneration similar to that of irreversible shock. The appearance of heart block early after clamping of the umbilical cord, its subsequent disappearance, and a progressively falling systolic blood pressure, pulse pressure, cardiac output, and cardiac rate can be correlated with the time of total asphyxia of the animal. Electrocardiographic changes with deepening anoxia approximate those observed in adult animals and patients, but the progression of these changes and the development of diminished arterial blood pressure is slower in the newborn. Experiments were performed in which arterialized blood from a bubble oxygenator (Gollan type) was introduced into the umbilical vein of lambs delivered by cesarean section and not allowed to breathe. Umbilical artery blood was returned to the Signamotor pump and oxygenator so that a circulation similar to that of the animal in utero was maintained. Continuous aortic pressures and electrocardiographic tracings were taken, and serum CO_2, Cl, Na, K, lactic acid levels, and blood oxygen levels were taken intermittently.

Unless the perfusion was started soon after interruption of the umbilical arterial flows, when the lamb's aortic blood pressure was only moderately depressed, recovery of the circulation did not occur. In spite of arterialization of the aortic blood to over 85% oxygen saturation, cardiac function became progressively worse. Several animals died of cardiac irregularities which developed after the aortic blood was well oxygenated.

If the perfusion was started early when the aortic blood pressures were moderately high, almost immediate improvement in the cardiac function was observed, as evidenced by increased aortic blood flow and return of aortic pressures to high levels as well as improved electrocardiographs. Several animals were maintained on the pump oxygenator for as long as one hour without showing deterioration of the circulation. With cessation of the use of the pump oxygenator the animals rapidly developed shock and terminal anoxic electrocardiographic changes.

The role of shock in neonatal asphyxia will be discussed, and the above results will be discussed in relation to the possible uses of the pump oxygenator as an artificial placenta and as a device for resuscitation of the newborn.

Discussion

JOHN J. OSBORN, San Francisco: I think it is entirely appropriate that the meeting should end with a paper of Dr. Harned's, because this is a paper of the future. This is the dream which got me, and I think probably a good many other people, into the field of pump oxygenating about seven or eight years ago, as I remember, after discussion and being pressed by Dr. Dancis. This idea of building an artificial placenta, I think it is a dream which actually may be fulfilled in the not-so-very-distant future, but I do think that it has to wait until we have better pump oxygenators.

Dr. Harned did not discuss it, but I have a rather strong feeling from listening that a good deal of difficulty actually came from the fact that the pump oxygenator cannot be used without

* By invitation.

serious damage to the blood. I think that there are better pump oxygenators being built, and experimental work going on leads me to hope that a pump oxygenator which can carry circulation not for two or three hours but actually for several days may actually be not very far away.

SAMUEL KAPLAN, Cincinnati: It is my impression that many of the failures in this series of experiments were due to the pump oxygenator. I would disagree with the statement that many difficulties arise with bubble oxygenators after prolonged perfusions. We have been able to successfully perfuse the total circulation of adult dogs for periods up to one and one-half hours. As pump oxygenators are developed, their use in the treatment of neonatal asphyxia will be of interest.

GEORGE S. HUSSON, Syracuse, N. Y.: I think that Dr. Kaplan probably has the fortune to have a bubble oxygenator that does work. At a recent meeting in Baltimore of some cardiac surgeons and cardiologists, the problem of open-heart surgery with the bubble oxygenator was thought to be related to the flow rates. Now, whether Dr. Kaplan's bubble oxygenator can give us these high flow rates and perform what he just described may be related to the amount of time that he can perfuse. However, in general, the bubble oxygenator has not been able to sustain circulation for long periods of time, as Dr. Osborn has just mentioned.

ROBERT E. COOKE, Baltimore: We became interested in this problem a relatively short while ago because of the confusion in the use of the terms anoxia, asphyxia, and ischemia, which are used almost interchangeably. I think that these studies put in proper perspective the tremendous importance of the circulation, which has been neglected by many physiologists who are involved in the problem of newborn breathing. The level of oxygen in the arterial blood may be relatively unimportant for the survival of the person after a period of anoxia, whereas the condition of the circulatory system may be of tremendous importance in the survival of the person. There are relatively few data that are worth while in relation to this aspect of the problem of neonatal asphyxia.

M. GROSSMAN, San Francisco: Are any data available on blood hemolysis during the prolonged experiment?

H. S. HARNED JR., New Haven, Conn.: Dr. Grossman, we did not measure the plasma hemoglobin levels. There was fairly marked hemolysis from these experiments.

I think, Dr. Osborn, you highlighted a very important point in that the oxygenator used here is not the answer by any means. We are actually very pleasantly surprised by how well it seemed to work and how well it seemed to work on other animals, with use of a very small volume of blood for priming. The limiting factor with membrane oxygenators and some of the other types is that they require far more blood for priming than we wanted to use in this particular experiment.

Papers Read by Title

Blood Destruction and Regeneration in Erythroblastosis Fetalis. FRED H. ALLEN JR. and LOUIS K. DIAMOND, Harvard.

Blood production at the time of birth in erythroblastosis has been calculated in 198 cases by multiplying hemoglobin concentration by the frequency of immature red cells. Nucleated red cells were added to the reticulocytes, since the nucleated red cells can constitute a fairly large proportion of the young forms in severely anemic erythroblastotic babies. It is assumed as a working hypothesis that red cell destruction in erythroblastosis is random and that many of the immature cells may be caught and destroyed before they can mature. Thus the count of reticulocytes and nucleated cells was revised upward by calculating how many had been destroyed and adding these in. The formula used for correcting the young cell counts was $\log_e \frac{1}{1-R}$, where R is the observed frequency of reticulocytes plus nucleated red cells. This formula assumes that blood production and blood destruction are equal at the time the measurement is made and that reticulocytes and nucleated cells are no more and no less likely to be caught in the hemolytic process than mature cells.

Averages of blood production values calculated in this way showed a consistent relation to babies' hemoglobin concentrations, with higher values (more blood production) in the more anemic babies. But there was a very great scattering of the individual values. Babies with high hemoglobin included many with very rapid blood production as well as many with low production. The latter obviously had a mild hemolytic process. The former obviously had been producing blood rapidly to compensate for a severe hemolytic process, and these are the babies who are in greatest danger of developing kernicterus, because the seriousness of their difficulty can easily be overlooked at the beginning, when they have high hemoglobin levels. As expected, there were many babies with marked anemia in spite of rapid blood formation, and all intermediate stages of anemia were seen in babies with rapid blood production. The same variability in anemia was present in babies with relatively low blood production, suggesting that some of the very anemic ones were responding poorly to a relatively mild hemolytic process.

Usually it is not clear why anemia has developed in a case of erythroblastosis fetalis, since one

usually has seen many other babies with apparently similar severity of hemolytic process but no anemia. The question of paramount importance in relation to the problem of erythroblastotic stillbirth is what the dead baby lacked that the liveborns appear to have been well supplied with. There are various possibilities suggested by information already available.

Phonocardiography: A Diagnostic Aid in Congenital Heart Disease. M. L. A. AUERBACK* and MAURICE SOKOLOW,* California. Introduced by FRANCIS SCOTT SMYTH.

Heart sounds and murmurs, in more than 100 children with congenital heart lesions, were recorded with a highly sensitive, well-damped phonocardiograph machine which was built by the Research and Development Laboratory of the University of California. The diagnosis was established in almost all cases by cardiac catheterization, surgery, angiography, or necropsy. The phonocardiographic observations were correlated with the clinical and hemodynamic findings.

Data obtained reveal that (*a*) various common uncomplicated congenital heart lesions produce murmurs that are almost always constant in configuration and timing; (*b*) "atypical" murmurs in such conditions as ventricular septal defect and patent ductus arteriosus may be produced by the disturbed pressure relationships between various heart chambers and great vessels, secondary to pulmonary hypertension, variations in flow, etc.

Slides were shown to illustrate some of the findings in detail: (1) the configuration of the murmurs of pulmonic and aortic stenosis, showing that, although both are diamond-shaped, that of aortic stenosis ends or decreases abruptly before the second sound, whereas the murmur of pulmonary stenosis ends at the aortic component of the second sound and is followed by a small delayed pulmonic component that is progressively smaller with increasing right venticular pressure (more severe pulmonic stenosis); (2) the typical pansystolic murmur of ventricular septal defects, becoming converted into a decrescendo systolic murmur and ending before the second sound, as the right ventricular pressure mounts; (3) the effect of pulmonary hypertension on the characteristic "machinery" murmur of patent ductus—the diastolic murmur gradually shortening to disappear finally as the pulmonary artery pressure rises; (4) examples of the variation in systolic murmurs found in atrial septal defect and the important auscultatory clue to the diagnosis provided by the wide splitting of the second heart sound.

Many pediatricians are not fully aware of the information that can be learned from auscultation in congenital heart disease. Study of the auscultatory findings will provide valuable clinical data that may make diagnosis possible at the bedside.

Epileptogenic Foci in the Basal Ganglia of Children with Convulsive Disorders. H. W. BAIRD III,* E. A. SPIEGEL,* and H. T. WYCIS,* Temple. Introduced by JAMES B. AREY.

Significant improvement in a child with tuberous sclerosis following the elimination of a subcortical focus by the stereotaxic method has already been reported. The demonstration of subcortical seizures and the subsequent electrolytic coagulation of the affected area and its pathways have been possible in five children. In addition, the five patients reported at the present time had failed to respond to drug therapy and were seriously retarded in their motor and mental development. All had more than one type of seizure. The commonest form was the minor motor seizure, which has been described by various authors as "infantile myoclonic," "salaam," "akenitic," "propulsive petit mal," etc.

Various time relationships between the pallidal discharges and those found in the scalp electroencephalographic tracing could be established cortical foci appears to be the pallidum; in some instances the amygdala was also involved. Following surgery there has been significant improvement in all but one of the children, who have been observed for periods of from three months to over two years. The observations suggest that the pallidum is a site not only where corticofugal epileptogenic impulses may synapse but also where epileptogenic impulses may originate.

Infantile Gastrointestinal Disturbances: A Pilot Study and a Design for Research. GIULIO J. BARBERO, DAVID RIGLER,* and JOHN A. ROSE,* Pennsylvania.

Infantile gastrointestinal symptoms, such as vomiting, feeding difficulties, and wide fluctuation in the number of bowel movements, have constituted and ill-defined and vexing problem as to cause and management in pediatric practice. It is the usual experience to find both irritability and sleep disturbance associated with this group of symptoms.

In the past few years, various etiologies have been suggested. Among these are milk allergy, "non allergic" intolerance of the gastrointestinal tract to dietary components, faulty feeding techniques, immaturity of the infant gastrointestinal tract and problems of tension in the mother-infant relationship. Some have considered that several of these factors may operate together.

Previous reports have noted improvement from nonspecific formula changes, removal of the infant from his environment, and even the use of the tranquilizers. Many still maintain the idea of spontaneous remission suggested by the title, "three months' colic."

* By invitation.

The rising number of such cases in pediatric practice, coupled with the confusion in etiology and management, suggested the need for clinical investigation of more rigorous nature than has been attempted.

In order to arrive at an adequate design for such a study, a pilot study was organized which involved some 30 infants under 7 months of age.

These infants were admitted to the Children's Hospital of Philadelphia at the height of symptomatic intensity and family distress. Observations of infant behavior and symptomatology were made for 48 hours with no other investigation attempted. After this initial period, necessary laboratory, radiologic, and motility studies were made. The infants were discharged and followed up at intervals of three to six weeks.

At the time of admission and during the 48-hour period of study, infant and mother were separated. Initially and during the 48-hour period, intensive psychiatric interviews were held with the mother. In general, the purpose of such interviewing was to develop the picture of the maternal psychic economy in relation to the mothering role. Specifically, an attempt was made to elicit any material which could interfere with maternal perception of the infant and his needs.

The findings of this pilot study seem of sufficient significance both to report and also to serve as a basis for a more elaborate and controlled design.

Some of these findings are reported. Of the 30 infants, 29 showed either complete symptomatic remission or considerable improvement during their hospitalization. The unimproved infant was confirmed as having pyloric stenosis by subsequent surgery. It is of interest that the vomiting of two infants showing esophageal hernias subsided almost completely. The majority of the infants showed deceleration of the rate of growth between birth and time of admission when compared with the average. Symptoms listed in order of frequency were vomiting irritability, changes in number and appearance of stools, feeding difficulties, and sleep disturbance. In most cases the symptoms reported were as fully initiated during the second neonatal week. Most infants had experienced multiple changes of formula, often with several days of symptomatic remission following the change. In some instances, there was symptomatic remission following the initial interview in the study. These cases were not hospitalized until exacerbation.

In all instances after discharge the mothers reported considerable to complete improvement. This was roughly validated by acceleration in the growth rate of the infant.

Interviews with the mothers suggested that a strong correlation existed between maternal tension and significant events relating to the mother's own emotional development and the psychic economy of the marriage and her pregnancy.

These findings will be reviewed in more detail and specific case illustrations of the various points given. The paper will conclude with some brief remarks about the current status of the proposed study.

Radiography of Prematures: A Practical Technique of Correlation with Respiratory Distress. WILLIAM ALLEN BAUMAN* and JEROME NADELHAFT,* Columbia. Introduced by William A. Silverman.

Technical difficulties and lack of normal standards in the past have hampered roentgenographic evaluation of premature infants. A planned study was undertaken utilizing portable x-ray equipment and a radiolucent platform within the incubator. Minimal disturbance of the patient was assured.

Quantitative clinical respiratory distress data were recorded simultaneously with each of several x-ray examinations of 104 premature infants during the first 72 hours of life. Gradation for pulmonary reticular-granular pattern of each film against known standards was accomplished by an experienced radiologist without clinical information about the patient. (Controls consisted of representative films of 1000 normal newborns and 7 with necropsy-proved hyaline membranes.)

Twenty-eight patients died under 14 days of age. Twenty-five were autopsied; ten had hyaline membranes. All 25 had the usual additional pulmonary abnormalities of prematures.

Reticular-granularity associated with respiratory distress was correlated significantly ($P < 0.01$) with death and also with autopsies in which hyaline membranes were noted. However, despite this correlation, from this study one cannot confidently conclude that x-ray abnormalities predict outcome.

An Anatomical Study of Circulatory Adjustment at Birth—Normal and Dilated Ductus. WILLIAM A. BLANCO and ANDRÉ NODOT,* Columbia. Introduced by DOROTHY H. ANDERSEN.

There are no available numerical data on dimensions of ductus arteriosus (D. A.) and aorta of the human newborn. Similarly, criteria of "normal" and "pathological" states are lacking.

In 208 stillborns, newborns, and infants, the circumference of the great vessels at various levels and of the D. A. have been measured and their interrelationship has been studied. The ratio ascending aorta/left carotid remained fairly constant in various age and weight groups. The ratio ductus/left carotid (ductus index, D. I.) has shown wide variation and is considered to be a reliable index of the degree of contraction of the ductus. In stillborns a high D. I. was found ("dilated"

* By invitation.

ductus) with the exception of four babies who received intracardiac epinephrine. In newborns a histogram showed a bimodal distribution curve of the D. I. Analysis of the two tails on either side of the median evidenced two groups with two statistically different means: a "dilated" group, corresponding closely to the stillborn series, and a low D. I. ("contracted" ductus) group. Of 127 newborns dead during the first week, 34 had a "dilated" ductus with a smooth intima. Ninety-three "contracted" ducts were occluded by extreme folding or thrombosis in 72 cases and had a smooth patent lumen in 21. No significant difference in the incidence of anomalies of pregnancy or delivery or in the neonatal history was found between the "dilated" and "contracted" groups. However, four dilated ducts produced audible murmurs. The incidence of most of the main pathological diagnoses was similar in both groups; these included hyaline membrane disease, a condition which could conceivably be associated with overcirculation in the lungs. However, factors which might mechanically perturb the normal hemodynamics were present in 21 of the 34 "dilated" ductus cases (premature closure of foramen ovale, 1 case; diaphragmatic hernia with pulmonary hypoplasia, hydrothorax, and pneumothorax, 4 cases; tight coarctation of the aorta, 12 cases; coarctation and pulmonary hypoplasia, 4 cases). The incidence of coarctation of the aorta, as defined by a ratio ascending aorta/narrowed arch ≥ 1.5, was identical in both groups. If a ratio of 1.60 was used as the criterion of coarctation, the association with dilatation of D. A. was highly significant. These observations will be analyzed in the light of recent physiological data and may provide a basis for future clinicopathological correlations.

Protein-Binding Properties of Mono- and Diiodotyrosine as Compared with Thyroxin and Triiodothyronine. Robert M. Blizzard* and H. David Mosier,* Johns Hopkins. Introduced by Lawson Wilkins.

Certain goitrous cretins have been demonstrated to have iodinated tyrosines present in serum. Interest therefore was aroused in ability of these substances to be bound to protein. MIT^{131}, DIT^{131}, triiodothyronine[131], and *l*-thyroxine[131] were added to samples of hypothyroid plasma. Recoverable PBI^{131} was measured by resin column, TCA, and Somogyi techniques. BEI^{131} was also determined. Dialysis against 0.85 mg. NaCl per 100 cc. was performed for 72 hours. After the addition of DIT^{131}, resin and supernates and precipitates of PBI^{131} were extracted, chromatographed, and radioautographed to see if structural change accounted for different recoveries of PBI^{131}. Electrophoresis was performed at pH 8.6 with DIT^{131} plasma samples, and correlation of activity and protein fractions was determined.

Results are listed in the following tabulation.

	Carrier	PBI^{131},* %			BEI^{131},* %	Dialyzable,*
		Resin	TCA	Somogyi		
MIT^{131}	0	1.8	0.7	64.0	1.9	98.4
	+	2.3	1.3	69.0	4.8	99.2
DIT^{131}	0	3.0	0.5	72.4	3.4	98.2
	+	1.5	7.9	72.6	3.2	98.1
T^3	0	41.9	96.0	88.6	91.7	20.0
	+	27.8	95.4	88.5	88.2	18.7
T^4	0	82.0	100	95.4	95.6	2.2
	+	82.3	105	97.3	100.0	2.2

* Each is average of duplicate samples.

DIT^{131} was found intact in butanol extracts of resin, Somogyi precipitae, and TCA and Somogyi supernates, and therefore physical or chemical change did not account for different recoveries. Electrophoretic studies revealed DIT^{131} traveling primarily with the albumin fraction.

It is concluded that iodinated tyrosines probably are weakly bound to protein at an alkaline pH and can be partially recovered by the Somogyi precipitation. The finding in a goitrous person of a high Somogyi PBI^{131} and a low TCA or resin PBI^{131} suggests iodinated tyrosines in the serum, as found in patients with dehalogenase enzyme absence. In addition, a discrepancy of PBI^{131} done by alkaline precipitation and the BEI^{131} probably occurs in this type of goitrous cretinism as well as in some patients with thyroid carcinoma or Hoskimoto's thyroiditis.

Fluorescent Antibody Studies with Agents Isolated in Tissue Culture from Acute Infectious Hepatitis. Joseph D. Boggs,* Roland F. Mais,* I. W. McLean,* and Wilton Rightsel,* Northwestern and Parke, Davis & Company. Introduced by J. A. Bigler.

Several investigators have conjugated flourescent substances with antibodies, using this material as a method for identification and localization of the corresponding antigen. Following the method reported by Rightsel and associates (1956), an agent producing a cytopathogenic effect on a Detroit-6 tissue culture line was isolated from a patient with infectious hepatitis.

The total globulin fraction of convalescent infectious hepatitis serum was conjugated with 5-dimethyl amino-1-naphthalene sulfonyl chloride. Anti-human-globulin serum was similarly conjugated. Electrophoretic analysis established an actual chemical union with the fluorescent chromophore. The isolated agent in tissue culture was incubated with convalescent hepatitis serum. The serum was shown to inhibit CPE before and after conjugation. The tissue culture was then incubated with conjugated anti-human-globulin serum. Certain controls were similarly treated and will be

* By invitation.

discussed. A definite tagging by conjugated globulin was apparent in those cells with agent isolated from acute infectious hepatitis. There was no evidence of tagging in the control series. The implications of this procedure are discussed.

The Embryological Development of Liver Function. Robert L. Brent,* Walter Reed Army Hospital. Introduced by Leo Geppert.

Our present knowledge of embryonic development has been mainly derived from gross and histological descriptions of normal and abnormal embryogenesis. The histological appearance of a developing organ may not correspond with its functional capabilities. Therefore it might be rewarding to examine the physiological and metabolic status of fetal tissues at different periods of gestation.

In our laboratory, this approach was applied to fetal liver development. Three parameters of liver function were studied at various times of gestation: (1) enzymatic content of liver tissue, (2) serum protein concentrations, (3) excretory function as measured by the ability of the fetal liver to excrete radioactive rose bengal.

Transaminase activity was measured in whole embryos and placentae (aged 11 through 22 days) and dissected fetal livers (aged 14 to 22 days). The appearance or release of active enzyme was not correlated with liver weight or growth rate. Irradiation of fetal livers did not alter the transaminase activity, although the embryos were irradiated at a time which considerably altered the morphological appearance of the liver.

Concentrations of serum proteins were measured by electrophoretic analysis on the 18th through the 22d day of gestation. As term approached, there was a gradual rise in serum albumin concentration. This reflected the changing contribution of the fetal liver toward serum protein production.

Lastly, the liver's ability to concentrate radioactive rose bengal was measured on the 17th through the 22d day of gestation. The dye was injected intravenously, and the liver and other fetal organs were dissected out at various times after injection. The amount of radioactivity per milligram of fetal tissue was determined. At the 11th and 18th day of gestation the ability of the liver to concentrate rose bengal was poor, but it improved as term approached. Even so, the newborn rat did not have the same functional capacity to excrete rose bengal as the adult rat.

Although the gross weight of the liver has a smooth exponential growth, its separate functional capacities have very different developmental patterns. Some mature early and others late—some are sensitive to radiation and others are not. It appears that many of the functional deficiencies of the premature and term infant may be better understood if the functional growth of the embryo can be more thoroughly investigated.

The Ammonia Content of the Gastric Juice in the Newborn Infant. Raymond L. Clemmens* and Samuel P. Bessman, Maryland.

The ammonia in the gastric juice arises from urease action on the urea which diffuses into the gastric secretion. This urease is not a normal animal enzyme but is produced by bacteria in the stomach. Measurement of gastric ammonia is, therefore, a gauge of active or recent bacterial growth in the stomach. Since this is the case, it represents a simple method for detection of infection in the stomach of an infant.

Excessive ammonia in the blood produces a variety of central nervous system symptoms. The venous ammonia content of newborns has been determined and has been found to vary between greater limits than are found in adults. The possible contribution of gastric ammonia production to venous ammonia will be considered.

Newborns 2 hours to 24 hours of age were studied. The ammonia level urease activity and pH of gastric contents were measured and simultaneous blood levels of ammonia were assayed. Determinations were also made on patients with erythroblastosis fetalis.

In general, ammonia is absent from the gastric juice of the newborn infant. It appears rapidly and is accompanied by the appearance of urease activity. Certain deviations from this pattern will be discussed with regard to the concurrent venous level of ammonia and the possible role of ammonia in the presence of symptoms of cerebral dysfunction in the newborn. The possible contribution of enterogenous ammonia in the pathogenesis of kernicterus will be discussed.

Data on methods, both oral and parenteral, for lowering blood ammonia content will be presented. Oral arginine and asparagine are effective in stimulating metabolism of ammonia as measured by its disappearance from the blood.

Factors in Growth and Development of Hospitalized Premature Infants. Flora Cherry,* William T. Newsom,* and M. Louise Pesut,* Tulane and Charity Hospital, New Orleans. Introduced by R. V. Platou.

Interpretation of growth and development patterns of premature infants during their initial hospitalization is of great importance in relation to care procedures, nutrition, illness, and socioeconomic factors. This is a report on the growth and development during the hospital stay of over 300 Negro prematures ranging in birth weight from 700 to 2000 gm. (9 below 1000 gm.) born at Charity Hospital in New Orleans, from August, 1955, to May, 1957.

* By invitation.

During this period, standard routines of care were provided all infants in this study so as to clarify specific variations in growth as a function of birth weight or maturity as the more obvious variables. On the basis of such care, serial weight, head, chest, and length measurements are graphically recorded in an effort to establish standards for comparison with future alterations of routines in our nursery. Simple analysis is made as to average duration of hospitalization, extent of neonatal weight loss, average time taken to regain birth weight and, average birth and weekly measurements. Special study is made of the weekly caloric intake and its relation to the weight curve. Mortality and morbidity figures are presented for the period embraced in the study.

Hypoglycemia of Infancy Induced by Amino Acids. W. A. COCHRANE,* Toronto. Introduced by A. L. CHUTE.

Infantile hypoglycemia with convulsions is not uncommon, and in the majority of cases the etiology is unknown. Recently Cochrane, Payne, Simkiss, and Woolf described four cases of hypoglycemia in infants in which the lowering of the blood glucose level was precipitated by the oral administration of the amino acids L-leucine and isovaleric acid. There was no effect noted when the amino acids were given to normal children. From the records of The Hospital for Sick Children, Toronto, 11 cases of idiopathic infantile hypoglycemia have been investigated by noting the effect clinically and on the blood sugar following the administration of casein hydrolysate and/or L-leucine by mouth. Blood sugar estimations were done 15, 30, 45, and 60 minutes after ingestion following a fasting blood sample. In four cases, all infants, a significant lowering of the blood sugar occurred, resulting in generalized convulsions in two cases. Serial EEG changes were noted following the administration of L-leucine in one case. L-Trypotophane produced no effect on the blood sugar in two cases. L-Leucine was also given intravenously to several rabbits and rats, with a lowering of the blood sugar in the latter group, but a slight elevation occurred in the rabbits tested.

Treatment of two of the "amino acid hypoglycemia" cases consisted of increased carbohydrate intake, 45 minutes after feeds, and clinical and blood sugar levels were compared to those found with treatment with steroids.

No effect was noted on the blood sugar in the remaining seven cases, which were all over the age of 5 years and were now asymptomatic. The effect of L-leucine on the blood sugar of two hypopituitary dwarfs is now being studied. One patient with juvenile diabetes mellitus was given large doses of L-leucine, with no particular effect on the blood sugar. The cause of the lowering of blood sugar by L-leucine remains unknown, but several possibilities will be discussed.

It is suggested that infants responding to L-leucine or amino acids should not be fed a high-protein high-carbohydrate diet as has been advocated in the past.

Respiratory Studies in Cystic Fibrosis of the Pancreas. C. D. COOK, P. J. HELLIESEN,* L. FRIEDLANDER,* L. L. KULCYZYCKI,* H. SCHWACHMAN, and S. AGATHON,* Harvard.

In persons with cystic fibrosis of the pancreas the extent of their respiratory involvement is most frequently the determining factor in their disability and survival. In the present investigation, lung volumes and the mechanics of respiration have been determined 63 times in 53 persons with this disease. The patients ranged in age from 1 to 31 years. The data have been compared with similar studies done on a control group of 53 "hospital normal" persons of comparable age and size.

The lung volumes have been measured with spirometry and a closed-circuit helium dilution technique. The mechanics of respiration have been calculated from intra-esophageal pressures as indices of intrapleural pressure changes and simultaneous measurements of respiratory volume changes. Compliance is expressed as the ratio of tidal volume to the change in intraesophageal pressure measured between points of no flow (millimeters per centimeter H_2O). Respiratory resistance is expressed as the ratio of total pressure change to corresponding total flow change between points of equal volume approximately midway in inspiration and expiration (centimeters H_2O per liter per second).

Although the severity of the disease process ranged from very mild to extremely severe, 81% of those diagnosed as having cystic fibrosis were found to have significant alterations in one or more of the above respiratory parameters. Most striking changes were observed in the residual volume and the resistance to air flow, which averaged, respectively, 62% and 85% more in the cystic fibrosis patients than in the normal persons of the same height. The compliance was significantly although less strikingly reduced (average was 37% below normal). As a result of these changes, the calculated work of pulmonary ventilation was moderately to markedly increased in many of the patients.

The residual volume data are consistent with the clinical, x-ray, and pathological evidence of emphysema observed in this disease. The increase in resistance to air flow provides a quantitative measurement of airway obstruction, which is at least in part due to excessive secretions and may also be due to bronchoconstriction. The interstitial

* By invitation.

fibrosis observed by x-ray and on pathological examination is reflected in the changes in compliance. Thus, the physiologic data are consistent with the important pulmonary manifestations of the disease. However, in the individual case, a comparison of the pulmonary-function studies with the patient's clinical condition suggests that, while there is a general correlation, pulmonary-function studies are necessary for accurate appraisal. It is also apparent that such studies may aid in the evaluation of various therapeutic measures. In three of the persons with increased air-flow resistance, measurements were made before and after the administration of a bronchodilator, isoprotereno (Isuprel). A response was noted in one patient, but no significant change found in the other two. The implications of these findings will be discussed.

The Use of Cineradiography with Image Intensification in Clinical Research. DAVID B. COURSIN,* HERBERT K. COOPER,* and F. ALLAN HOFMANN,* Lancaster, Pa. Introduced by SYDNEY S. GELLIS.

The development of electronic means for amplifying the intensity of the light image produced by x-rays has opened a number of new horizons for study in clinical research. This advance now permits minimal amounts of radiation to be used for seconds at a time, with resultant excellent visibility of parts under study. The Phillips Image Intensifier increases the light intensity of routine fluoroscopy level by one thousand times with resultant improvement of human eye visual perception from 2% to 3% to almost 100%. Selected positioning of the patient with respect to components of the apparatus further provides magnification of specific parts under study by a factor of two and one-half times.

Recordings of function and activity during observation can be made on 16 mm. film running as rapidly as 120 frames per second. Additional provisions have been made for simultaneous sound recordings that are synchronized with the picture sequence. The application of this technique particularly to the problems of respiration, speech, and cleft palate will be discussed. Other applications in the fields of angiocardiography, gastrointestinal and genitourinary motility, cardiac activity, and joint motion will be commented upon.

The Occurrence of Hypoproteinemia in Infantile Gastroenteritis. DAVID BAIRD COURSIN,* CHARLES H. KURTZ,* and VIRGINIA BROWN,* Lancaster, Pa. Introduced by SYDNEY S. GELLIS.

Infantile gastroenteritis with its exacerbations, debilitating effects, and death continues to be a major pediatric problem despite progress in antibiotics and fluid-replacement therapy. The present study was carried out in 50 infants with diarrhea in an effort to find additional factors that might be of importance in controlling these problems. Ultramicro blood chemistry studies were performed on admission and at intervals during the illness: pH; CO_2; Cl; Na; K; Ca; P; BUN; hematocrit; albumin globulin, and α-, β-, and γ-globulin. A standard accepted therapeutic regimen was followed, with use of intravenous fluids, antibiotics, and gradual resumption of selected dietary intake.

Clinical evaluation and laboratory studies were correlated to evaluate the degree of illness as mild, moderate, or severe. Electrolyte changes were those customarily seen in the course of diarrhea. However, the most interesting observations were those of measurable depletion of plasma proteins, which coincided with the severity of the disease and the patient's progress. The more severe and chronic the diarrhea state, the more marked were the losses of body proteins, as shown by the low levels of plasma proteins as well as the decrease in volume content of the fluid spaces of the body. Electrolyte replacement solutions improved the chemical and volume picture but further diluted the protein concentrations. It was therefore found expedient to use plasma and protein hydrolysates in an effort to overcome this situation. The recognition of these protein needs has provided a better understanding of the complications of gastroenteritis and has pointed up their requirements in therapy. Data will be presented.

Transport Across the Placental and Red Cell Membrane. JOSEPH DANCIS, New York.

A major function of the placental and cell membranes is the selective transfer of nutritive materials. A quantitative comparison of performance was made with use of an amino acid (histidine) and a carbohydrate (xylose). Varying amounts of the test substance were infused into a pregnant guinea pig for a half-hour, and then the increases in concentration in the red blood cell and fetal plasma were measured. A definite correlation was demonstrable. At high levels of histidine there was evidence for saturation of a transport mechanism in the placenta.

The Value of Microscopic Examination of the Stool for Extracellular Starch in the Diagnosis of Disease States. MURRAY DAVIDSON* and CHARLES H. BAUER, Cornell. Introduced by IRVING SCHULMAN.

The syndrome of mild celiac disease, or starch intolerance, as defined by Andersen, is predicted upon a number of clinical and laboratory deviations from the normal. It has become commonplace, however, to make this diagnosis in any young child with recurrent or persistent diarrhea whose stool is simply found to contain free extracellular

* By invitation.

starch. The purpose of this study was to evaluate the reliability of this test.

Approximately 200 fresh stool specimens, collected at random from more than 70 children free of gastrointestinal symptoms, were examined microscopically after staining with strong iodine (Lugol's) solution. The results show that an appreciable percentage of normal formed stools contain free starch. Specimens were obtained from the rectum emptied prematurely by administration of hypertonic phosphate solution enemas and by digital removal of stool. The starch content of more than 80 samples collected in this way is significantly elevated when compared with control samples.

Clinical studies in which neomycin was administered to normal children, supplemented by in vitro experiments with starch solutions and bacterial cultures, indicated that contact with normal colonic flora could influence the amount of extracellular starch found in stool specimens. The implications of these findings and their relationship to the chief laboratory tool currently employed in the diagnosis of the syndrome of starch intolerance will be discussed.

Serum Electrophoretic Studies in Cystic Fibrosis of the Pancreas. CAROLYN R. DENNING,* Columbia. Introduced by DOROTHY H. ANDERSEN.

Quantitative estimates of the serum protein components were made by paper electrophoresis in 40 children with cystic fibrosis of the pancreas. These were compared with normal controls.

With use of arbitrary criteria, the patients were divided into groups according to the severity of their clinical disease. Whenever possible, studies were repeated on the same patient at intervals to determine the effect of changes in the clinical status on the various protein fractions. Further correlations were made between serum γ-globulin concentrations and zinc turbidity determinations done on the same sera.

The serum γ-globulin and α-2 fractions were found to be significantly elevated and the serum albumin fraction significantly depressed in patients classified as moderately to severely ill. These discrepancies became more pronounced with progression of the disease process. Zinc turbidity determinations afforded reliable indices of γ-globulin concentrations in the serum.

It is believed by the author that periodic inspection of the serum protein fractions as assessed by paper electrophoretic methods permits evaluation of the rate of progress of the pulmonary disease in cystic fibrosis of the pancreas and is of some prognostic value.

* By invitation.

Serum Penicillin Levels Following Intramuscular Injections of Benzathine Penicillin G in Children with Inactive Rheumatic Fever. ANTONI M. DIEHL,* ROSWITH I. LADE,* IRVIN SNYDER,* and TOM R. HAMILTON,* Kansas. Introduced by C. ARDEN MILLER.

Benzathine penicillin G, 1,200,000 units given by intramuscular injection every 28 days, is accepted as a means of preventing streptococcal infection in rheumatic subjects. This investigation was undertaken to determine the relation of the concentration of penicillin in the serum to age, weight, and sex of children with inactive rheumatic fever and to correlate nose and throat cultures for hemolytic streptococci and titers of antistreptolysin O with serum concentrations of penicillin.

Ninety-six patients between the ages of 4 and 20 years were included in the study, which ran from September, 1956, through February, 1957. All were in a quiescent phase of their rheumatic process. They received 1,200,000 units of benzathine penicillin G every 28 days by intramuscular injection. Just before each injection the nose and throat were cultured for the presence of hemolytic streptococci of Lancefield Group A and blood specimens were drawn for determination of the titer of antistreptolysin O and the concentration of penicillin in the serum. The Sarcina lutea cup-plate inhibition method of Welch was employed in assaying the penicillin concentration of the serum.

The number of determinations was as follows: concentrations of penicillin, 204; cultures of the nose and throat for hemolytic streptococci, 491; titers for antistreptolysin O antibody, 490. One culture yielded a Group A Streptococcus which was not typable. The patient from whom this culture was obtained had no associated increase in titer of antistreptolysin O. One other patient had a significant (two-tube) rise in antibody titer; this was not preceded by a positive culture. The levels of penicillin in the serum could be determined with certainty in 81% of the specimens; concentrations ranged from 0.008 to 0.125 units per milliliter. In 19% of the specimens penicillin could not be assayed with certainty.

The mean concentration of penicillin in the serum of females was three times higher than it was in males. The concentration of penicillin in serum of females increased as their age and weight increased from the 10th to the 17th years, inclusive. There was no increase of penicillin concentration with age or weight in males. The differences of concentration of penicillin in the sera of the two sexes were statistically significant ($P < 0.01$) from the 10th to the 17th year, inclusive. No significant difference of serum concentration of penicillin was found in obese and thin girls of the same age. Explanation for the differences between the two

sexes is inapparent at the present time. During the period of study there were no recurrences of rheumatic fever in either sex.

Pituitary Deficiency with Serum Electrolyte Abnormalities Simulating Adrenocortical Insufficiency with Normal Circulating 17-Hydroxycorticosteroids: A Case Study. GEORGE N. DONNELL* and NEIL N. LITMAN,* Southern California. Introduced by MERL J. CARSON.

Idiopathic pituitary insufficiency in infancy, manifested by growth retardation and secondary target-gland involvement, is extremely rare. Well-documented cases occurring in the first year of life are those associated with either anencephaly or pituitary and adrenal atrophy, both of which are incompatible with life after 1 or 2 weeks of age. In older children pituitary insufficiency has been described with some frequency. The majority of these cases are the result of destructive lesions of the pituitary gland. The clinical syndrome of growth retardation with or without evidence of hypothyroidism has been attributed to pituitary dysfunction, although unequivocal evidence for this diagnosis is often lacking. The role of the adrenal gland under these circumstances is not well understood.

We have observed an infant with marked somatic retardation in whom signs of secondary hypothyroidism and hyposadrenocorticism were present. Although there were no stigmata of cretinism, evidence for secondary hypothyroidism was based on bone-age retardation, low PBI, and low I^{131} uptake with marked increase of the uptake following administration of thyrotropic hormone. Adrenal cortical insufficiency was suggested by the presence of chronic dehydration, hyponatremia, hyperkalemia, absolute eosinophilia, and excessive urinary loss of sodium and chloride. Paradoxically, the circulating 17-hydroxycorticosteroids (Nelson-Samuels) were high normal and stimulation with corticotropin (ACTH) caused a supernormal response.

To explain the excessive urinary loss of Na, one has to postulate either a segmental adrenal defect involving mineralocorticoids or a renal tubule defect. Renal-function tests revealed intact glomerular and tubular function, ruling out the syndrome of "salt-losing nephritis" as a cause of the renal loss of sodium.

Attempts to correct the electrolyte disturbance by employing various hormonal preparations (desoxycorticosterone acetate [Doca], corticotropin, 8-1 corticosteroids) were unsuccessful. Large salt loads were necessary before the serum electrolyte levels returned to normal. Short periods of Na restriction in order to evaluate mineralocorticoid excretion (aldosterone) resulted in the clinical and blood chemical findings of Addisonian crisis. Study of this infant suggests pituitary deficiency manifested by a deficiency in thyrotropic hormone and growth hormone as well as segmental adrenocortical insufficiency involving mineralocorticoids. The clinical and laboratory studies leading to the above conclusions will be discussed.

Biochemical Indications of Potassium Intoxication as Studied in the Rat. ALEXANDER N. DRESCHER,* PATRICIA A. MEARA,* MARY TERRY,* and NATHAN B. TALBOT, Harvard.

This study was undertaken to determine the effects of heavy potassium loading on the content and concentration of potassium in the extracellular and intracellular compartments.

For this purpose, 150 gm. rats were placed on a complete diet providing 100 mEq. of potassium and 50 mEq. of sodium per square meter per day. Potassium was added to this diet in 500 mEq. per square meter per day stepwise increments at approximately five-day intervals. A 3 KCl:1 $KHCO_3$ mixture was used as a dietary supplement in order to avoid the acidosis and concomitant changes in body water and potassium distribution produced by simple KCl loading. Serum potassium concentration showed an upward trend with increasing intake. Fifty per cent of the animals died within four days after a potassium intake of 2100 mEq. per square meter per day had been reached, indicating that the ceiling of body tolerance for potassium at this sodium intake was between 1600 and 2100 mEq. per square meter per day. Eight animals surviving the L. D_{50} dose were killed and analyzed individually.

In these animals, values for the total water content of the total carcass and of cardiac muscle were normal. Intracellular and extracellular volumes also were normal for the whole carcass, but intracellular water was slightly increased at the expense of extracellular water in the hearts. Total body and cardiac muscle potassium contents were normal, as were intracellular potassium concentrations. Extracellular accumulation of potassium was evidenced by serum potassium concentrations ranging from 4.1 to 12.9 mEq. per liter (mean, 8.0 mEq. per liter). Serum pH and CO_2 were normal. Extracellular chloride concentration was elevated comparably to the rise in extracellular potassium concentration. No mean changes in extracellular or intracellular sodium content or concentration were found.

Considering the individual carcass analyses, there was no correlation between serum potassium concentration and the intracellular potassium values. In the cardiac muscle, in which intracellular potassium concentrations were found to be in the low normal range, a positive correlation of low slope was observed between the concentration of potassium in extracellular and in intracellular water.

* By invitation.

Electrocardiograms taken immediately prior to killing revealed changes which correlated with both extracellular and heart intracellular potassium concentrations. These changes were similar to those observed following acute parenteral potassium loading.

It is concluded that the primary mechanism of potassium homeostasis is adaptive excretion. When potassium intake exceeds maximum excretory capacity, under conditions of normal extracellular acid-base balance, only minimal intracellular storage of potassium occurs. Consequently, the surplus accumulates in the extracellular compartment and hyperkalemia follows. In this study elevation of serum potassium was the significant biochemical evidence of potassium intoxication. Electrocardiographic tracings also appeared to be a dependable index of potassium intoxication. The fact that these electrocardiographic changes occur in association with an abnormal elevation in extracellular rather than in intracellular potassium concentration raises intriguing questions.

The Role of Carbon Dioxide, Oxygen, and pH in Initiating Respiration in 256 Newborns. DONAL DUNPHY,* LOREN G. MACKINNEY,* and VIVIAN PESSIN,* Buffalo and Pittsburgh. Introduced by MITCHELL I. RUBIN.

Hypoxia and CO_2 have each been proposed and rejected as the stimulus to newborn respiration. Contradictions in conclusions can be attributed to the difference in time at which blood samples were drawn and to the small number of observations.

This report is an analysis of cord blood chemical data from 256 full-term infants. Deliveries were observed and the intervals from birth to the initiation and to the establishment of respiration were timed with a stop watch.

Cord vein values for O_2 content and saturation, pH, CO_2 content, and pCO_2 were found to be consistent with those in the literature when the time of sampling was taken into account. The importance of considering the exact time of sampling was demonstrated by showing the rapidity of changes in chemical values during the first hours of life.

Although values for these infants were within the range for stimulus of respiration, it does not necessarily follow that they actually did stimulate. The absence of correlation between chemical values and time of breathing of individual children would negate the assumption that a particular value acted as a stimulus. The question of whether these physiologically possible stimuli are actual stimuli was explored by examining regressions of O_2 content, pH, and pCO_2 on intervals between delivery and the first gasp and between delivery and the establishment of respiration.

* By invitation.

Pregnanediol Excretion During the Menstrual Cycle of Adolescent Girls. WALTER R. EBERLEIN and ALFRED M. BONGIOVANNI, Pennsylvania.

A simplified apparatus has been devised which enables the small hospital laboratory to employ paper chromatography for quantitative purposes. With use of this apparatus, a highly sensitive and accurate method has been developed for the assay of pregnanediol excretion in the urine of adolescent girls, to determine whether menstrual irregularities at this age signify anovulatory menstrual bleeding. Five young menstruating girls were investigated by assaying urine serially over a five-week period for follicle-stimulating hormone, estrogens, and pregnanediol. Daily basal temperatures were also obtained.

The chromatographic apparatus and the new method for determination of urinary pregnanediol excretion will be described. The results of the above study will be presented and discussed in relation to normal variations in adolescent development and hypothalamic-pituitary control of ovarian function.

Epidemic Diarrhea in Premature Infants Caused by a Cytopathogenic Enteric Virus. HEINZ F. EICHENWALD, ALEXANDER ABABIO,* ALBERT M. ARKY,* and ALLEN P. HARTMAN,* Cornell.

During the summer of 1956, an outbreak of epidemic diarrhea occurred in a premature nursery, affecting 12 out of 21 infants. The affected infants ranged in age from 6 to 46 days and in weight from 1000 to 2200 gm. Clinically, the diarrhea was mild, lasting from one to five days, and was not accompanied by fever or vomiting.

Ten of the twelve infants with diarrhea showed a cytopathogenic agent in their stools and developed antibodies to it. The two sick infants from whom no virus was isolated showed a significant rise in antibody titer to the same agent. None of the well infants excreted the virus, nor did they develop immunity to it.

The virus readily causes cytopathogenic changes in monkey kidney monolayer cultures, but initially it did not take well in human amnion or liver cells. It is nonpathogenic to suckling mice, nor can it be neutralized by Coxsackie, adenovirus, or poliovirus antisera. The agent possesses the biological properties of the enteric cytopathogenic human orphan (ECHO) group. By suitable cross-neutralization tests, it was shown to be identical to the D-3 virus of Ramos-Alvarez and Sabin (*Ann. New York Acad. Sc.* 67:326, 1957).

Subsequent to the outbreak in the premature nursery, cases of diarrhea occurred among five infants on another floor of the same institution. These patients ranged in age from 1 week to 2 months. The D-3 virus was isolated from the

stool of each of these patients, and increases in antibody titer were demonstrated.

Use of Hypothermia for Relief of Valvular Pulmonic Stenosis Under Direct Vision. MARY ALLEN ENGLE,* GEORGE R. HOLSWADE,* HENRY P. GOLDBERG,* and FRANK GLENN,* Cornell. Introduced by NORMAN KRETCHMER.

Reduction of body temperature to (30 C) lowers metabolic requirements by half and permits interruption of circulation for as long as eight minutes with full recovery. At this temperature blood pressure is near normal, and danger of ventricular arrhythmia is slight.

Use of hypothermia has increased the safety and the efficacy of surgery of one of the common congenital malformations of the heart, isolated valvular pulmonic stenosis. While circulation to the body is interrupted for three to four minutes, the pulmonary artery is opened so that the cone-shaped pulmonary valve may be seen and incised out to the valve ring along the lines of the fused commissures. This procedure has resulted in greater relief of obstruction than has been possible by closed or blind techniques.

The signs of severe valvular pulmonary stenosis and their regression after surgery were studied in the first six patients operated on for this condition and observed for 6 to 18 months postoperatively. Preoperatively all had obstruction to pulmonary blood flow of serious degree, with right ventricular systolic pressures ranging from 150 to more than 200 mm. Hg. Two were in heart failure and two were cyanotic. The mortality rate has been zero, and the success rate, 100%. Regressions following pulmonary valvotomy of one form of subvalvular stenosis due to hypertrophied right ventricular musculature in two of the patients was noted. In all patients the electrocardiograms changed from a pattern of right ventricular hypertrophy and "strain" to normal. Pressures in the right ventricle and pulmonary artery have become normal in those studied by cardiac catheterization one year after valvotomy.

Red Blood Cell Destruction, Production, and Splenic Sequestration in Patients with "Mild" Hereditary Spherocytosis. MARION ERLANDSON,* IRVING SCHULMAN, VINA RUGGERO,* and CARL H. SMITH, Cornell.

Persons with hereditary spherocytosis may present with little or no anemia. These persons have been thought to have a mild form of the disease or even a carrier state. Decisions concerning management or the advisability of splenectomy have been made difficult because of the scant evidence of physical debility resulting from the presence of the disease and because of increasing evidence that splenectomy in childhood may predispose to severe or even fatal infection. Therefore, in a group of seven patients with mild hereditary spherocytosis (hemoglobin concentrations 10.5 to 13.5 gm/100 cc.) quantitative studies designed to characterize the presence of a hemolytic syndrome were carried out.

By means of red cell tagging with Cr^{51}, blood volumes, red cell survival times, and rates of red cell production and destruction were determined. In addition, organ localization of the erythrocytes was determined by surface scintillation counting. Rates of red blood cell destruction ranged from 4.4 to 9.8 times the normal rate. No correlation was found between rate of destruction and hemoglobin concentration or red cell mass. Rather, presence or absence of anemia was determined by the particular balance obtained between the rates of destruction and the rates of red cell production. The latter values ranged from three to six times the normal rate. No constant pattern was found among family members in the several family groups included.

From the studies of splenic uptake of Cr^{51} activity, presumably reflecting splenic sequestration of tagged red blood cells, it appeared that the degree of splenic activity varied directly with the rate of red blood cell destruction and not with spleen size.

It is apparent that the high hemoglobin values in "mild" hereditary spherocytosis exist in the face of a severe hemolytic process, compensated by increased marrow activity. These patients are susceptible at any time to the manifestations and complications described in the "severe" form of congenital spherocytic anemia. No evidence has been found for the existence of a true carrier state. The implications of these findings in terms of management will be discussed.

The Effect of Corticotropin on the Renal Clearance of Tyrosine and Para-Hydroxyphenylpyruvate in Premature Infants. DONNELL D. ETZWILER,* HELEN MCNAMARA,* NORMAN KRETCHMER, and S. Z. LEVINE, Cornell.

Para-hydroxyphenylpyruvate is formed during the metabolism of phenylalanine and tyrosine and is oxidized to acetoacetate in the normal person. Levine and co-workers were the first to describe the spontaneous excretion of *p*-hydroxyphenylpyruvate and tyrosine in the urine of premature infants fed a high-protein diet. Further studies by these investigators revealed that the addition of ascorbic acid (vitamin C) to the diet eleminated this tyrosyluria. The administration of corticotropin (ACTH) produced a similar result, but a longer period of time was required. The site of this action was not known.

* By invitation.

The renal clearance of tyrosine and *p*-hydroxyphenylpyruvate has been studied in premature infants to determine the role of the kidney in tyrosyluria. Tyrosine and creatinine were measured colorimetrically, and *p*-hydroxyphenylpyruvate was determined enzymatically and spectrophotometrically. The concentration of tyrosine in the blood was 25 to 40 mg/100 ml., and the concentration of *p*-hydroxyphenylpyruvate, 2 to 6 mg/100 ml. The renal clearance of tyrosine in these premature infants is approximately 10% of the clearance of *p*-hydroxyphenylpyruvate.

The clearance of these substances was measured before and after the administration of corticotropin to infants with tyrosyluria to determine the mechanism for disappearance of the tyrosyluria. The data indicate that the site is not the tyrosine-oxidizing system of the liver but rather an increase in the renal clearance of tyrosine and *p*-hydroxyphenylpyruvate.

The Effects of Corticotropin on Magnesium Metabolism in Nephrosis. FRANCIS X. FELLERS* and ROBERT SCHWARTZ, Harvard.

One of the frequent unexplained complications of steroid therapy in children with nephrosis has been the occurrence of convulsions and tetany. During a systematic exploration of the serum electrolytes, attention has been directed toward serum magnesium alterations in children treated with corticotropin (150 mg. per square meter per day for 10 days).

In a group of 37 clinically well children with nephrosis with serum proteins greater than 6.0 gm. per 100 cc. the mean serum magnesium level was 2.14 mg. per 100 cc. (range, 1.82-2.78 mg. per 100 cc.) in 46 observations, while in 30 patients with active disease (serum proteins under 5.5 gm. per 100 cc.) the mean serum magnesium was 1.96 mg. per 100 cc. (0.92-2.58 mg. per 100 cc.). Sixteen of the latter group (35% of observations) were below the range of control nephrotic patients (<1.82 mg. per 100 cc.). No relationship between serum protein concentration and serum magnesium values was found in the children with active disease (S. P. <5.5 mg. per 100 cc.). Magnesium levels in edema fluid compared simultaneously with serum from four patients gave an average ratio of 0.81 in 11 observations.

Serial serum magnesium determinations in seven courses of corticotropin given to six children indicated a significant fall in five instances and no change in two. The decline in serum magnesium concentration began prior to the onset of diuresis in two patients. Minimal values were attained after onset of diuresis between the 9th and 12th days (1.02-1.64 mg. per 100 cc.).

Urinary magnesium excretion was followed daily in four corticotropin courses. Prior to therapy, magnesium excretion was 5 to 30 mg. per day, while during the first three days of therapy values declined (three of four courses) to less than 15 mg. per day and then rose before the onset of diuresis to attain maximal values of 40-100 mg. per day during diuresis.

In order further to elucidate the mechanisms involved in the hypomagnesemia, a detailed balance study was carried out in one patient over 35 days. In addition to the decline in serum magnesium and rise in urinary magnesium during diuresis, an increased fecal loss of magnesium was observed between the third and ninth days of corticotropin and coincided with the decline in serum magnesium.

No neurological complications developed coincident with the hypomagnesemia in these patients.

Functional, Anatomical, and Biochemical Alterations of the Brain Resulting from Experimental Hypernatremic Dehydration. LAURENCE FINBERG, CHARLES LUTTRELL,* and HENRY REDD,* Johns Hopkins.

Patients with hypernatremic dehydration have been noted to have disturbances of central nervous system function resulting in alterations of consciousness, ataxia, spasticity, and convulsions. A few such patients have had subarachnoid hemorrhages, and recently we have observed seven infants with subdural effusions complicating hypernatremic dehydration.

Experiments were designed to study the effect on the central nervous system of hypernatremic dehydration experimentally produced in kittens. Seventeen kittens weighing between 250 and 500 gm. were injected intraperitoneally with 3.5 cc/100 gm. of a solution containing 1500 mEq. per liter of Na, 1000 mEq. per liter of Cl, and 500 mEq. per liter of HCO_3. Litter mates were studied as controls. Following injection the animals were observed at regular intervals for 24 hours in metabolism cages, during which time no water or food was given and urine was collected. The weight of the surviving animals prior to killing was about 10% below the preinjection weight. At 24 hours the 11 survivors were killed by exsanguination; autopsies were performed with particular attention to the brain, and specimens of muscle and brain were obtained for chemical analysis. The tissues were analyzed for water, fat, Na, K, Cl, N, and Mg contents. Sera were analyzed for CO_2 content, Ca, P, and total osmolar concentration in addition. Urines were analyzed for osmolarity, Na, K, and Cl.

Shortly after the injection all the animals manifested lethargy, hyperirritability, marked ataxia, and tremulousness; some of them convulsed, and six became stuporous and died within 24 hours.

* By invitation.

Gross examination of the brains showed a marked increase (over controls) of CSF which was either xanthochromic or blood tinged. In 13 animals there were subdural hemorrhages noted; most of these were 1 to 2 mm. in diameter, but a few ranged up to 5 mm. in size. Some of these animals showed small intracerebral hemorrhages as well. No gross hemorrhages were noted in any other organs of the body, including the abdominal viscera. Three control animals subjected to simple thristing and four injected with an equivalent volume of an isotonic Na solution showed none of the clinical or anatomical changes described above.

The results of the chemical analyses on 11 surviving animals were compared with the findings of analyses on 9 uninjected litter mates killed coincident with injection of the experimental animals. The serum water Na concentration in the experimental animals ranged from 164 to 222 mEq. per liter, and serum osmolarity ranged to 420 mosm. per liter. The chemical anatomy findings of the tissues studied were compared, with use of the chloride space as an approximation of the extracellular water and the remaining water being considered as cellular water. Water was found to have been lost from the extracellular water and cellular water in equal percentage increments from both muscle and brain. On the other hand, there was considerable increase in cellular sodium in muscle but not in brain. There was no significant change of cellular K per 100 gm. of fat-free dry solids in either tissue. These data give a further indication of metabolic dysfunction in the brain as compared with muscle, since no intracellular sodium accumulates in brain cells to aid in achieving osmotic equilibrium following increased osmolar concentration in extracellular fluid. An explanation for this phenomenon could be that the osmolar concentration in the brain cell is increased through the breakdown of complex ions within cells. Such an occurrence would be expected to be associated with gross disturbances in cell function.

In summary, the clinical features of CNS involvement in hypernatremic dehydration have been simulated in the experimental animal; moreover, the anatomical findings are similar to those seen in some severely ill patients. Finally, the chemical anatomy of the experimental condition suggests a possible mechanism for the severe functional disturbance.

Potassium Binding Site of Mitochondria and of Mitochondrial Fragments. J. L. GAMBLE JR., Johns Hopkins.

Relationships between potassium binding and oxidative phosphorylation have been observed in mitochondria and mitochondrial fragments. In the case of the intact mitochondria evidence that the processes are linked derives from considerations of the dependence of both functions upon the relative intactness of the mitochondrial structure.

Experiments with a preparation of mitochondrial fragments that contains the enzymes of oxidation and of phosphorylation have revealed that activity of the electron transport system can affect both the stability of the potassium-binding site and the rate of the potassium exchange reaction at the site. This influence does not appear to be dependent upon the presence or active synthesis of ATP, but it is abolished by dinitrophenol. The probability of an association between the potassium-binding site and the enzymes responsible for coupling oxidation to phosphorylation is discussed.

Long-Term Studies of Andrenalectomized Monkeys (Macaca Mulatta). ROBERT D. GAUCHAT * and CHARLES D. MAY, Iowa.

Studies of at least 70 totally adrenalectomized monkeys have been published since the early observations of Kahn in 1912. Within this literature, the report of Stewart, Salmon, and May is unique in that it provides long-term observations of an adrenalectomized monkey maintained in good metabolic balance without recourse to exogenous adrenocorticosteroid support. The present paper reports additional observations of 22 monkeys studied for as long as 1500 days after physiologically complete adrenalectomy.

Previous workers have experienced difficulty in maintaining adequate nutrition following removal of monkeys' adrenals. After accustoming animals preoperatively to a liquid formula designed to provide adequate daily allowances of known essential nutrients, it has been possible to continue this liquid diet, supplemented with NaCl and $NaHCO_3$ during the postoperative period, thereby achieving satisfactory nutritional and electrolytic balance in most cases. Antibiotics and cortisone have been given routinely during the first five postoperative days and then discontinued. Thereafter, antibiotics and steroids have been briefly administered only at times of major systemic infection associated with acute hypoadrenocorticism or for purposes of special short-term studies.

The physiologic completeness of adrenalectomy has been confirmed in each instance by typically Addisonian responses to administration of insulin and corticotropin, as well as by the need for additional salt.

Deaths have occurred from 11 to 840 days after adrenalectomy. Necropsies have revealed no remnants of adrenal tissue. The oldest survivor has now been adrenalectomized for more than 1500 days.

Numerous observations concerning various aspects of the role of the adrenal glands in normal metabolism have been made and will be docu-

* By invitation.

mented. Growth and sexual maturation continue at normal rates after adrenalectomy. Voluntary intake of fluids and output of urine are not quantitatively altered by adrenalectomy. During periods of anorexia associated with infection, adrenalectomized monkeys are able to utilize fat as a source of energy and can concentrate the urine. Quantities of 17-ketosteroids excreted per day in the urine are reduced but not completely eliminated in either sex by adrenalectomy. Adrenalectomized animals given diphtheria toxoid become Schick-negative, and titers of complement in the sera of adrenalectomized monkeys are not significantly different from the titers of intact animals. On the basis of observed frequency of crises and duration of survival, the data suggest that male animals adapt to the adrenalectomized state with greater facility than do female animals, but this adaptability was not affected by orchidectomy in the one male animal in which this has been done. Two male animals, but no females, have ultimately tolerated withdrawal of added salts from their formula without ill effect. Decreased ability to maintain a stable concentration of glucose in the blood appears to be the most significant factor in governing viability of adrenalectomized monkeys.

A method for maintaining adrenalectomized monkeys in good nutritional balance for long periods of time without steroid supplementation has been achieved. Studies of this closely related species may shed additional light on the complex problems of human adrenocortical physiology. Observations already made cast serious doubt upon current hypotheses concerning the role of the adrenal cortex in various normal and pathologic processes.

Radioactive Rose Bengal: An Aid in the Differential Diagnosis of the Jaundiced Infant. LEO J. GEPPERT and ROBERT L. BRENT,* Walter Reed Army Hospital.

Radioactive rose bengal is an iodinated fluorescein dye, in which the iodine is replaced with I^{131}. Taplin has recently introduced it into adult medicine to aid in determining the state of liver function in certain liver diseases. Because the dye is specifically absorbed and excreted by the parenchymal cells of the liver, once administered it can only appear in the bile and gastrointestinal tract unless the rose bengal is degraded in the body. Although its usefulness in adult medicine is somewhat limited, the possibility of utilizing rose bengal in differentiating biliary atresia from other causes of jaundice was investigated. The clinical course, punch or surgical biopsy, and surgical exploration have yielded the information upon which we evaluate an infant's jaundice. With these and other laboratory techniques, it still was not uncommon to have the jaundice clear in a child previously diagnosed as having biliary atresia.

Thirteen infants with jaundice of questionable etiology were given, intravenously, 4μc. or less of radioactive rose bengal. In some cases a superficial counter was placed over the liver away from the gallbladder to record the rate of uptake and excretion. In other cases the feces and urine were collected for several days following the administration of rose bengal and the amount of radioactivity was determined. Combination of these methods were used in still other cases.

In those children with true biliary atresia no radioactivity was obtained in the feces and all the iodine was eventually excreted in the urine. In other forms of liver disease rose bengal was handled abnormally but nonspecifically. Two infants who were thought to have biliary atresia clinically and by biopsy had relatively normal rose bengal excretions. The condition in both these infants eventually cleared, substantiating the validity of the test results.

The techniques of the test will be described, with an explanation of the difficulties of biopsy for diagnosis of biliary atresia in a child with long-standing jaundice.

The Mechanism of Hyperlipemia in the Nephrotic Syndrome. DAVID GITLIN and PAUL A. M. GROSS,* Harvard.

The pathogenesis of hyperlipemia in the nephrotic syndrome has been shown to be due both to an increased production of low-density lipoproteins and to a decreased fractional rate of conversion of these lipoproteins to those of higher density. The absolute increase in the concentration of low-density lipoproteins is responsible for the lactescence of serum in nephrosis.

Clinicians have recognized for many years a rough correlation between the severity of hypoalbuminemia in this disease and the severity of hyperlipemia as determined by cholesterol levels. Albumin is necessary for the conversion of low-density lipoproteins to those of higher density by accepting the fatty acids split off during the conversion; in vitro, the accumulation of fatty acids inhibits the conversion process. These facts and other experiments have led to the concept that the failure of conversion is due to hypoalbuminemia. However, the correlation between hypoalbuminemia and hypercholesterolemia is not absolute, and, consequently, a study was made of lipoprotein lipase in the nephrotic syndrome, since this enzyme is directly responsible for initiating the conversion process. It was found that patients with the nephrotic syndrome show varying degrees of deficit of this enzyme and that accumulation of low-density lipoproteins in this disease could be attributed to this deficit.

* By invitation.

In conclusion, there are at least two components, lipoprotein lipase and albumin, which are essential to the conversion of low-density to high-density lipoproteins, with the subsequent delactescence of lipemic serum.

Isolation of Cytopathogenic Agents from Cerebrospinal or Vesicle Fluid of Patients with Herpes Zoster or Varicella. ELI GOLD* and FREDERICK C. ROBBINS, Western Reserve.

The mode of spread, the pathogenesis, and the relationship between varicella and herpes zoster infections may be better understood if recently available tissue culture techniques are applied to the problem. Weller and Stoddard, employing a human embryonic tissue culture system, observed the development of eosinophilic intranuclear bodies in tissue cells following inoculation with varicella or herpes zoster vesicle fluid.

In our laboratory identical cytopathic changes were observed in tissue cultures of monkey kidney, human amnion, or HeLa cells inoculated with vesicle fluid from patients with either herpes zoster or varicella. The changes in the cells were associated with the presence of eosinophilic intranuclear inclusion bodies. An agent producing the same cytopathogenic changes, including the intranuclear inclusion bodies, was obtained from the spinal fluid of a 6-year-old girl ill with herpes zoster involving the right eye and forehead.

The agents isolated were passed serially and withstood freezing and thawing. Serological studies concerning the relationship between herpes zoster and varicella will be described.

Validity of Traditionally Accepted "Normals" in Physiologic Study of Children: Some Physiologic Evidence of Child's Emotional Response to Blood Sampling. BRUCE D. GRAHAM, JOHN H. READ,* DAVID G. DICKINSON,* GEORGE R. DEMUTH,* M. U. TSAO,* and CHARLES SLOAN,* Michigan.

Children of various age groups were sampled for blood gas determinations at various conditions of "rest." Continuous measurement of alveolar pCO_2 before, during, and after arterial puncture was also performed on many of these patients. Blood obtained by arterial puncture was analyzed for hemoglobin oxygen saturation, blood pH, CO_2 content, plasma oxygen tension, and (calculated) carbon dioxide tension.

An initial group of 10 normal children (ages 6-13) was sampled in the treatment room off the ward. All had been briefed on the procedure; they were apprehensive but none to the point of serious physical objection. A few sobbed before the procedure, but all were quiet during the procedure and for the two minutes before the sampling.

Data obtained indicate a tremendous variation in plasma pO_2 levels, blood pH, and calculated arterial blood pCO_2. Hemoglobin saturation was always very constant. The relationship of this apparent disparity to the oxygen dissociation curve will be discussed.

In an attempt to obtain more "controlled" normals a series of persons was then observed daily with the infrared CO_2 analyzer, measuring the alveolar pCO_2 level, until a steady, constant, near "normal" level was obtained. Arterial samples for blood gas determinations were then obtained, with concomitant alveolar gas measurements being made.

Still another group, of adult volunteers, and a further group, of polio patients, were sampled in various states of respiratory activity. The results of these studies are compared in terms of normality, and therapeutic implications will be discussed.

Observations on the Carbohydrate Metabolism of Premature Infants: I. The Blood Sugar During Fasting; II. The Response to Epinephrine; III. The Response to Glucagon. DENMAN HAMMOND,* California. Introduced by F. S. SMYTH.

Observations concerning the behavior of the fasting blood sugar level from the 6th hour of life up to the 18th day have been made on a group of 35 healthy premature infants of birth weights from 1300 to 2400 gm.

Sixteen of these infants have been subjected to epinephrine-tolerance tests done serially in order to compare the glycogenolytic response during three periods of physiological adaptation: during the period of physiological hypoglycemia, shortly after the institution of feedings, and again during the period of consistent weight gain. Ten infants have been subjected to glucagon-tolerance tests done serially during the same three periods.

In the absence of symptoms, the hypoglycemia of newborn and, especially, prematurely born infants has been considered physiologic. This "physiologic hypoglycemia" has been ascribed to depletion of glycogen stores by an organism dependent almost entirely upon carbohydrate sources for energy production.

Evidence will be presented that the hypoglycemia of the premature infant exists despite adequate stores of glycogen.

Maternal and Fetal Formation of Glycogen from Dietary Glucose and Fructose. DENMAN HAMMOND,* with the Technical Assistance of ARLENE MURPHY and PHEBE MILLER, California. Introduced by F. S. SMYTH.

Comparison of blood sugar and urinary sugar levels during comparable infusions of glucose and fructose have indicated that fructose is removed from the blood stream more rapidly; however, this

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is not due to increased renal losses. It has been demonstrated that the rate of glycogen formation in the liver of fasting rats is greater after a fructose meal than after a comparable glucose feeding. The pathways of fructose utilization have not been completely elucidated. Whether its rapid disappearance from the blood stream is due to an increased rate of oxidation, of glycogen formation, or of fat formation or whether it may be stored in organs other than liver are questions which have been raised and need further investigation.

This study includes observations of the effect of diets high in fructose and glucose on liver glycogen of pregnant and lactating rats and their offspring.

Virgin adult female rats of the Long-Evans strain were bred to fertile adult males. Those showing sperm in the vaginal secretion following breeding were placed in identical cages in groups of three. All animals were of comparable age and weight and had been identically fed. A purified diet was compounded free of carbohydrate, and to this basic diet were added fructose, glucose, or an equal mixture of the two to the extent of 60% of the diet by weight. One of the three test diets was offered to each group of rats daily in weighed amounts; distilled water was offered ad lib. Animals were individually weighed three times weekly.

Experiment A.—After 20 days of gestation, groups of animals from each test diet were killed by sudden cranial trauma, immediately followed by opening the abdomen to secure a blood sample from the still pulsating aorta. The uterus of each was excised in toto, from which fetuses were delivered. The fetuses were then eviscerated; the livers were removed intact, pooled, weighed, homogenized, and sampled. Maternal livers were removed intact, weighed, homogenized, and sampled. Placentas were cleaned of attached membranes, pooled, weighed, homogenized, and sampled.

Blood samples were analyzed for total reducing substance, fructose, and nonfermentable reducing substance. Glucose was calculated by difference. Maternal livers, pools of fetal livers, and pools of placentas were analyzed for glycogen.

Experiment B.—Other pregnant rats fed each of the test diets were allowed to deliver their litter spontaneously. Litters and mothers were weighed the morning after littering, and litters were equalized. Mothers were continued on their assigned test diet throughout a 20-day period of lactation, at which time mothers and litters were killed; specimens were obtained and analyzed as in Experiment A.

Results.—There were no significant differences in weight gain during pregnancy, the number of fetuses, or the weight of fetuses. Average maternal liver weights were slightly greater in the fructose-fed than in those fed the combined glucose-fructose diet. Liver weights of those fed glucose alone were less than those fed either fructose-containing diet.

Maternal liver glycogen content was nine times greater in fructose-fed animals than in those fed glucose alone. Glycogen content of those fed the glucose-fructose mixed diet was five times greater than those fed glucose alone.

The average fetal liver glycogen content was greatest in those from glucose-fed mothers and least in those from fructose-fed mothers. Placental weights and glycogen content were not significantly different from group to group. Placental glycogen content was very small in relation to liver glycogen.

Differences between groups after a 20-day lactation period were similar but of less magnitude. Liver glycogen content was two and a half times greater in livers of mothers fed fructose than in those fed glucose and was two times greater in those fed the mixed diet than in those fed glucose alone.

Blood sugar studies revealed higher concentrations of total blood reducing substance and blood fructose in the sucklings of mothers fed the fructose diet. There were no significant differences in blood glucose levels.

The Influence of Sedative Drugs on Renal Hemodynamics and Electrolyte Excretion.

L. Leighton Hill* and C. W. Daeschner,* Baylor. Introduced by Russell J. Blattner.

It is often desirable to use sedative drugs in the preparation of children for renal hemodynamic studies. However, no data are available to indicate the extent to which the commonly available soporific agents influence renal function in children. Studies in dogs reveal that both morphine and the barbiturates depress glomerular filtration rate and renal blood flow.

The present studies were undertaken to determine the influence of three sedative agents (meperidine [Demerol], chloral hydrate, and phenobarbital) on glomerular filtration rate, renal blood flow, renal vascular resistance, water excretion, and the clearance of sodium and potassium. In addition, the influence of chloral hydrate and phenobarbital on tubular excretory capacity for *p*-aminohippurate was measured.

Each patient served as his own control, following which the drug to be studied was given. As soon as the subject showed evidence of sedation the clearance periods were repeated. Glomerular filtration rate was measured by the clearance of inulin, and renal plasma flow, by the low-level clearance of sodium *p*-aminohippurate.

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All sedative agents studied produced some degree of reduction in renal blood flow. The most marked reduction was seen following meperidine, and the least, with phenobarbital. Glomerular filtration rate was decreased following meperidine but not after phenobarbital or chloral hydrate sedation. Mean blood pressures were not significantly influenced by the degree of sedation utilized. Renal vascular resistance was increased by meperidine and chloral hydrate but not by phenobarbital. Tubular excretory capacity for sodium *p*-aminohippurate was reduced by all of the sedative agents studied in this manner. The rate of water excretion showed a sharp initial reduction following meperidine administration, with a tendency to return toward control values by the end of the study period. Water excretion was similarly influenced by phenobarbital and chloral hydrate, but to a lesser extent. The clearance of sodium and potassium was reduced to a greater extent by meperidine than by phenobarbital or chloral hydrate.

Study of Relations Between Homeostatic Limitation in Renal Capacity to Conserve Water and the Physical, Intellectual, and Emotional Development of a Child with Renal Diabetes Insipidus. DONALD A. HILLMAN,* OLCAY NEYZI,* PHILIP PORTER,* ANNE CUSHMAN,* and NATHAN B. TALBOT, Harvard.

The subject of these studies was a 15-month-old boy with vasopressin (Pitressin)-resistant, or nephrogenic, diabetes insipidus. When first seen he had the height, weight, and skeletal, mental, and emotional status of a normal child between 3 and 7 months of age. When he ate adequate amounts of a diet of ordinary composition, his obligatory fluid requirements amounted to approximately 10 liters per square meter per day. It was found, however, that he could not drink fluid at a rate greater than 9.5 liters per square meter per day without becoming physically exhausted. This physical fatigue ceiling on his capacity to take fluids therefore made it impossible for him to eat enough food of ordinary solute content to fulfill his normal caloric requirements. As a result of this, he had the physical characteristics of a hypocaloric dwarf.

Detailed records were made of the patient's every activity during the course of several representative eight-hour periods. Analysis of these data showed that while the patient was taking a normal diet for age, he needed to drink every 25 minutes and void every 50 minutes. This meant that he could sleep only in snatches and that he needed to spend much of the day as well as the night getting his total sleep requirement in the form of short naps. As a consequence, the patient was so preoccupied with drinking, voiding, and sleeping that he had little opportunity for normal social contact, play, or learning. It was therefore not surprising that the patient showed gross retardation of mental and emotional as well as physical development.

In an effort to minimize the disturbances outlined above, he was placed on a diet designed to fulfill his caloric and other dietary needs without creating a heavy demand for water. This resulted in a reduction in the time occupied in drinking, voiding, and sleeping to approximately normal levels and a corresponding increase in the time devoted to playing and learning. Since discharge home on this diet, the child has gained and grown physically and developed intellectually and emotionally at a markedly accelerated pace.

It is concluded from the foregoing that one can define the effects of a limitation in homeostatic capacity upon the daily life of a child in approximate quantitative-qualitative terms. It is believed that such information is of value in defining the patient's therapeutic needs, in planning ways to fulfill them, and in judging the efficacy of therapy.

The Antibody Response of Infants and Children to Vi Antigen and Influenza Vaccine. NANCY HUANG* and MAURICE LANDY,* Temple. Introduced by ROBERT H. HIGH.

Children with chronic diseases are generally believed to be more susceptible to bacterial infections than are children without such diseases. This assumption has prompted us to carry out the present investigation. A total of 123 infants and children were included in this study; 78 were infants and children of average health, and 55 had various chronic diseases. All of the subjects were given a single injection of 0.04 mg. of Vi antigen administered subcutaneously and a single injection of 0.1 ml. of polyvalent influenza virus vaccine intracutaneously. Samples of serum were obtained prior to immunization and three weeks after the immunization of all subjects. The antibody titers to Vi antigen and to influenza viruses were measured in these samples of serum.

The results of the present study demonstrated that young infants of one year or less of age were inferior to older children in their capacity to produce antibodies to Vi antigen and to influenza vaccine. However, the development of antibodies in sick infants and children was not significantly different from that in infants and children of average health.

A significant antibody response was observed following a single injection of Vi antigen, which is a polysaccharide. In this respect these results were similar to those obtained previously in adults.

By contrast, the antibody response to influenza virus vaccine, which is believed to be a protein, probably represents a secondary response.

* By Invitation

Primary Pulmonary Obliterative Vascular Disease in Infants and Young Children. GEORGE S. HUSSON* and TYREE C. WYATT,* New York at Syracuse. Introduced by J. B. RICHMOND.

The pathologic physiology and histopathologic findings in primary pulmonary hypertension are briefly presented.

An attempt is made to distinguish between this type of pulmonary vascular problem occurring in the young from that in the older age groups. Although the clinical findings, laboratory data, and courses are similar in the two groups, data will be presented which suggest that they may represent two different entities in terms of pathogenesis and etiological factors.

Detailed studies with respect to the over-all histopathologic findings and the connective tissue components of the lesions found, especially certain aspects which have not been previously emphasized in young children, will be described.

Reversible Myocarditis Associated with Acute Infection in Infants and Children. GEORGE S. HUSSON,* O. ROBERT LEVINE,* and RUTH BILLIG,* New York at Syracuse. Introduced by JULIUS B. RICHMOND.

The more frequent use of the electrocardiogram in the pediatric age group has resulted in an increasing awareness of the involvement of the myocardium in various acute infections in infants and children. Serial electrocardiograms and x-rays can demonstrate the functional state of the myocardium during the course of various diseases.

Myocarditis, occurring as an apparently fatal complication of systemic infection, has been well documented in postmortem material by Saphir and others. However, the course of the disease in nonfatal cases of myocardial inflammatory disease has only recently been reported in the pediatric literature.

Several cases are presented in which definite myocardial changes were detected during the course of acute illness in infants and children and in which recovery occurred without sequelae. These have been documented by serial electrocardiographic and x-ray studies. Emphasis is placed on the criteria for recognition of this complication of acute illness in pediatrics and on its reversibility.

Kernicterus in Rats with Familial Jaundice. LOIS JOHNSON,* WILLIAM A. BLANC,* JEROLD F. LUCEY,* and RICHARD DAY, State University of New York, Columbia, and Vermont.

Kernicterus may be defined by the following four criteria: (1) neurologic abnormalities and jaundice prior to death; (2) canary-yellow staining of areas of gray matter in brain and spinal cord; (3) failure of the yellow stain to turn green after fixation in formaldehyde; (4) presence of nerve-cell damage and of intracellular yellow pigment on microscopic examination.

The above criteria are satisfied in homozygous newborn rats of a strain described by Gunn in 1937. In 1940 Molloy and Loewenstein showed that the defect lies in an incapacity of the liver to convert indirect bilirubin into its direct-reacting form and to excrete bilirubin. The disorder is similar to that observed in the children described by Crigler and Najjar. The rats, like the children, may die in the neonatal period. The survivors may or may not have nervous sequelae. Death occurs usually between 10 and 25 days after birth. This interval can be shortened by the injection of small amounts of bilirubin or sulfisoxazole (Gantrinsin) or sulfadiazine.

Moving pictures will be shown demonstrating motor incoordination. Color slides will show the gross and microscopic pathologic findings of the central nervous system.

Digitalis Intoxication in Infancy and Childhood. HOWARD A. JOOS and JOHN L. JOHNSON,* Southern California.

During a recent period of 27 months, the authors have observed 10 cases illustrating (*a*) frank digitalis intoxication, (*b*) at least twofold overdigitalization without symptoms or signs, (*c*) manifestations compatible either with toxic effects of digitalis or with specific or potentiating effects of underlying heart disease or associated illness, and (*d*) the problem of differentiating similar effects of intoxication with multiple cardioactive medications. Three patients died, the digitalis being a primary or contributing cause of death. This experience permits examination of circumstances under which overdosage occurs. It emphasizes that clinical symptoms and signs, as well as alteration in cardiac rhythm and other electrocardiographic manifestations, may differ in incidence and in character from those which might be expected in an overdigitalized adult cardiac population. As therapeutic advances enhance the survival of pediatric cardiac patients, the pediatrician may seek more familiarity with these differences and with the effects of pediatric disease, extracardiac as well as cardiac, on the manifestations of excessive digitalization.

In summary, clinical and electrocardiographic manifestations were not related to the size of the overdose. Two examples of probable digitalis intoxication occurred in children who received digitalis not in excess of maximum therapeutic doses commonly employed. The frequent occurrence of congestive heart failure as a manifestation of digitalis intoxication in early life emphasizes the need to consider this cause in the differential diagnosis of cardiac decompensation.

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Dehydration is cited as a possible factor contributing to the occurrence of digitalis poisoning or resulting from it, aggravating the intoxication. A wide variety of electrocardiographic disturbances in rhythm and conduction was demonstrated. First degree A-V block occurred in half the cases. Disturbances in A-V conduction and supraventricular arrhythmias were much commoner than abnormalities in ventricular conduction or rhythm. Ventricular arrhythmia was limited to the fatal cases, with one exception.

Digitalis therapy in infants and young children requires particular caution from all concerned. Whenever possible, the opportunity for error should be minimized by the use of standardized products, familiar to both physician and pharmacist.

Water Metabolism in Sickle-Cell Anemia. HANS G. KEITEL,* Jefferson. Introduced by WALLACE MCCRORY.

Almost all patients with sickle-cell anemia and about 70% of subjects with the sickle-cell trait have hyposthenuria (Keitel, Itano, and Thompson, 1956). The present report concerns the ability of the kidney to excrete water in these conditions.

The "free" water clearance (CH_2O), urea clearance (CM), and the urine and plasma osmolar concentrations were determined in 11 patients with sickle-cell anemia and in 8 subjects with sickle-cell trait, all of whom were given a standard water loading test. The "CH_2O" and urea clearance were normal in both groups, and in some patients with sickle-cell anemia they were supranormal. In three patients with sickle-cell anemia and an elevated plasma urea concentration the CH_2O was only slightly reduced whereas the urea clearance was 50% below normal.

Since there was evidence that the maximum ability to excrete water had not been attained during the standard water-loading test, enough 2.5% dextrose was administered parenterally to some of the sickle-cell anemia patients with a normal urea concentration of plasma to sustain a positive water balance of from 3% to 7% of body weight. Urine osmolar concentrations dropped as low as 16 (the lowest known value in man) and the "CH_2O" rose as high as 38 ml. per minute per 1.73 sq. meter and the urea clearance rose markedly.

It is concluded that the ability to excrete water and form maximally dilute urine is normal or enhanced in patients with sicklemia without urea retention, even though they have hyposthenuria. A possible explanation for these data is suggested by the finding of an elevated glomerular filtration rate in these patients.

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Idiopathic Infantile Agranulocytosis with Hypergammaglobulinemia. WILLIAM T. KNIKER * and THEODORE C. PANOS, Texas.

Two infants with persistent, fatal agranulocytosis have been studied recently. The clinical, laboratory, and pathological features closely fit the syndrome described by Kostmann, infantile genetic agranulocytosis (*Acta paediat.* 45, Supp. 105, 1956). Recurrent skin and respiratory infections began during the neonatal period, suggesting congenital origin of the disorder. No genetic or familial relationships could be identified, however. The cases likewise closely resemble those described by Janeway et al. at the 1954 meetings of the American Pediatric Society (*A.M.A. Am. J. Dis. Child.* 88:388, 1954), although their cases exhibited somewhat later onset, less severe neutropenia, and inconstant bone marrow findings.

The patients were admitted at 7 and 30 months of age, having experienced a steady succession of severe skin and respiratory infections since early infancy. There was unvarying severe neutropenia throughout their clinical course. Splenomegaly was prominent in both and hepatomegaly in one; there was no generalized lymphadenopathy. Repeated bone marrow examinations (unchanged by therapy) revealed a spectacular and consistent pattern of hypercellularity, pronounced myeloid left shift with maturation arrest at the myelocyte level, plasmacytosis, and eosinophilia. Serum paper electrophoreses revealed consistent excess of γ-globulin and decreased albumin. In one case, the administration of 50 ml. of γ-globulin over an eight-week period was without apparent benefit. Evidence for response to antigenic stimulation was inconclusive. Steroid therapy produced subjective improvement in both but did not affect significantly the bone marrow histology or the peripheral blood picture, the latter consistently showing complete neutrophilic agranulocytosis or severe neutropenia.

Because of the hypercellular bone marrow, splenomegaly, and failure of therapy to affect the agranulocytosis favorably, splenectomy was advocated. One patient was allowed to go home prior to surgery and died suddenly later, presumably of meningitis. The other case underwent splenectomy at the age of 8 months; the spleen was moderately enlarged (40 gm.), exhibited evidence of previous perisplenitis, and microscopically showed only chronic passive congestion. The patient died, in spite of massive specific antibiotic therapy, on the 14th postoperative day, with overwhelming sepsis due to Pseudomonas aeruginosa. Postmortem examination showed evidence of septicemia, a general hemorrhagic tendency, and no tissue plasmacytosis.

The cases under discussion could represent a congenital defect in marrow myeloid maturation with secondary hypergammaglobulinemia, in an

ineffectual attempt to bolster body defense. However, there may well be a more generalized defect of the reticuloendothelial system with agranulocytosis as a result of either a myeloid defect or manufacture of leucocyte agglutinins (as yet undetected) and production of excess γ-globulin. The evidence points to a primary defect in bone marrow cellular maturation; although no blood cell counts were obtained in the immediate neonatal period, the very early onset of symptoms suggests a congenital origin. Because of the absence of genetic factors and the hepatosplenomegaly, these patients apparently differ from Kostmann's cases. Because of the very early onset, the striking agranulocytosis, and constant bone marrow findings, these cases also apparently differ from those described by Janeway et al.

Serum Transaminase Activity in the Neonatal Period: Preliminary Observations in Jaundice of the Newborn Infant. SIMON KOVE,* STANLEY GOLDSTEIN,* and FELIX WROBLEWSKI,* New York and SLOAN KETTERING. Introduced by L. E. HOLT JR.

Measurements of serum transaminase have proven of value in the diagnosis of liver diseases in adults. It therefore seemed of interest to apply these techniques to the newborn infant.

Since no information, except for the brief abstract of Abelson et al. (*A. M. A. J. Dis. Child.* 92:512, 1956), was available on the serum transaminase at this time of life, it was first necessary to establish normal ranges. Studies were therefore made of serum glutamic oxaloacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T) activity in 63 normal newborn term infants for the first week of life. It was found that the range of activity for these two enzymes was somewhat greater than that of adults, in whom 5 to 45 units is considered normal. The range of activity for SGO-T was found to be 13 to 120 units. That for SGP-T was 12 to 90 units.

No relation was found between the degree of hyperbilirubinesia and the level of either enzyme activity, despite levels of serum bilirubin of 10 to 15 mg. per 100 cc. in 13 infants and 15 to 33 mg. per 100 cc. in 6 infants.

Observations were also made on a smaller number of infants with pathological conditions. The effect of hemolytic states on serum enzyme activity were studied by serial determinations in seven infants with erythroblastosis fetalis. In six of these the disease varied from mild to severe, and all had normal levels of enzyme activity. In the seventh infant the disease was of a most severe, fulminating character. The level of SGO-T activity in cord blood was 332 units and then dropped to the normal neonatal range over the next few days. The SGP-T activity remained at a normal level from birth.

Two infants with biliary obstruction were studied. In the first infant, one month of age, with the probable diagnosis of atresia of the bile ducts, the levels of enzyme activity rose slowly to 576 units for SGO-T and 450 units for SGP-T. In a second infant, with erythroblastosis fetalis, the levels of enzyme activity began to rise progressively, upon the development of the "inspissated bile syndrome at one week of age," from normal levels to 336 units for SGO-T and 272 units for SGP-T.

Conclusions.—Levels of serum transaminase activity up to about 120 units for SGO-T and about 90 units for SGP-T must be considered physiologic in the early neonatal period. Physiological hyperbilirubinemia of the newborn infant does not influence the levels of transaminase activity. Preliminary observations indicate that in hemolytic states the levels of serum enzyme activity are usually normal, although in the presence of very severe hemolysis SGO-T activity alone may be somewhat elevated for a short time. It would appear that in biliary obstruction there are moderate and sustained elevations of SGO-T and SGP-T activity up to about 600 units.

Tyrosine Metabolism of the Young: The Conversion of Phenylalanine to Tyrosine. GABRIELLE H. REEM * and NORMAN KRETCHMER, Cornell.

The conversion of phenylalanine to tyrosine is known to be the major pathway for phenylalanine metabolism. It has been shown by Jervis that the livers of persons with phenylpyruvic oligophrenia lack the enzyme necessary for the hydroxylation of phenylalanine to tyrosine. It was established that certain of the tyrosine-oxidizing enzymes were inactive in the livers of fetal rats and premature infants. In view of these findings, development of the activity of the enzyme responsible for the transformation of phenylalanine to tyrosine was studied in fetal, newborn, and adult rats.

The soluble fractions of liver homogenates were shaken at 35 C with L-phenylalanine, diphosphopyridine nucleotide, and nicotinamide for periods up to 120 minutes. Tyrosine formed from the phenylalanine was measured colorimetrically after precipitation of proteins.

It was found that the yield of tyrosine is minimal in the fetal liver and in the first 24 hours after birth. Under the conditions of these experiments only 0 to 0.1 micromole of tyrosine per milligram of nitrogen was formed in one hour. The ability to hydroxylate phenylalanine develops rapidly after the first day of life.

Preparations of liver from rats 4 to 12 days old formed 0.12 to 0.46 micromoles of tyrosine per

* By Invitation

milligram of nitrogen per hour. The level of activity in the liver of adult rats was 0.4 to 0.5 micromoles per milligram of nitrogen per hour, corresponding to the complete conversion of the added phenylalanine.

Acquired Hemolytic Anemia: 1. Use of Radioactive Chromium in Diagnosis; 2. Use of Mechlorethamine (Nitrogen Mustard) in Therapy. WILLIAM KRIVIT,* Minnesota. Introduced by ROBERT A. GOOD.

1. Despite the relative assurance of establishing the diagnosis of acquired hemolytic anemia with use of the Coombs sera, other diagnostic procedures have to be employed in a minority of patients. The cross transfusion studies utilizing radioactive chromium have provided an excellent adjunct to our diagnostic armamentarium.

The survival of normal erythrocytes when transfused into the patients under study have demonstrated an extracorpuscular defect. Conversely, the patient's cells, when injected into a normal person, have had a normal chromium cell survival.

By the use of this technique two patients with a negative direct and indirect Coombs test were diagnosed as having acquired hemolytic anemia. (The Coombs tests were done with trypsinized and acidified normal cells, in addition to the routine manner.) Both of these patients responded to cortisone, as was predicted from the chromium red cell survival studies. In the first patient who had normal cells transfused into him, Cr^{51} had a half-life of 6 and 10 days. Conversely, the patient's cells demonstrated an approximate half-life of 20 days when transfused into a normal person. The second patient was given normal cells tagged with radioactive chromium. The half-life of these cells was 10 hours; the immediate effect of the institution of cortisone therapy was graphically demonstrated by a dramatic change in the rate of hemolysis.

2. The treatment of acquired hemolytic anemia remains difficult and prolonged. Dacie, in his review, notes that 50% of the patients with idiopathic acquired hemolytic anemia will have a remission after splenectomy. A similar percentage of patients obtained relief of symptoms and signs with steroid therapy.

A 3-year-old girl who had a Coombs-positive idiopathic acquired hemolytic anemia had been unsuccessfully treated by splenectomy and exploration for accessory spleens. The severity of the hemolytic process had been decreased by massive doses of cortisone and corticotropin. The entire process, however, had been present for more than three and one-half years. Because of the marked hypercortisonism, mechlorethamine (nitrogen mustard) was given in an effort to interfere with reticuloendothelial function and possibly with antibody synthesis. A marked diminution in fecal urobilinogen and reticulocytes and increase in hemoglobin occurred. Subsequently the hemolytic anemia recurred and the mechlorethamine was given for a second and third time with similar results. Following the third course of intravenous mechlorethamine, the hemolytic anemia ceased and the Coombs test became negative. This patient had had normal values for one year.

The second patient was treated in a similar manner because of the excellent results obtained as above. The child had a splenectomy and had to be maintained for two years on steroid therapy. He has received three courses of mechlorethamine, and there has been a reduction in fecal urobilinogen, rise in hemoglobin, and reduction in reticulocytes after each course. During the third course of mechlorethamine a radioactive chromium study was done. We were able to demonstrate by the use of the red-cell-survival technique a reduction of the rate of hemolysis. However, there was a prompt return of the hemolysis four days after the administration of mechlorethamine, to the original rate of red cell destruction.

Studies of Cytotoxic Effect of Nephrotoxic Serum on Renal Tissue Culture Cells. CHARLOTTE T. LIU * and WALLACE W. MCCRORY, Pennsylvania.

We have found that rabbit anti-rat-kidney serum possesses factors that are cytotoxic for rat renal tissue culture. This in vitro cytotoxic effect appears to be related to the antibodies responsible for in vivo nephrotoxicity in rats and the titer of anti-kidney hemagglutination test measured by Boyden's technique. Other rat organ antisera were used in these same tests to determine the organ specificity of this effect. Sera lacking nephrotoxic effects and failing to show cytotoxicity had titers of anti-kidney antibodies of 1:640 or less. The potent nephrotoxic sera made by injecting rat kidney homogenate had antikidney titers above 1:10,000, produced severe renal disease in rats when a dosage of 0.4 to 1 ml. was injected, and showed unequivocal cytotoxic effects at 1:48 to 1:96 dilution of the sera in rat renal tissue cultures. The cytotoxic and nephrotoxic effects were removed by adsorption of the sera with rat kidney homogenates and kidney digest. The adsorption with organ digests, other than kidney, showed considerably reduced activity in this respect. The rat kidney cells grown in tissue culture were effective in neutralizing the potent nephrotoxic serum. The cytotoxic effect appears to be species-specific. While the nephrotoxic serum was toxic in the dilution of 1:96 in Long Evans rat kidney tubes, 1:24 in white rat kidney tubes, and 1:12 in DBA mouse kidney tubes, it was nontoxic in rabbit, monkey, and human kidney tubes.

* By Invitation

Sera from patients with nephritis and nephrosis possessing measurable anti-human-kidney antibody titers have been tested for evidence of cytotoxic effects for human renal tissue cultures, and findings will be discussed.

The Response of Bone to Alloxan-Diabetic Acidosis in the Rat. CHARLES C. LOBECK* and GILBERT B. FORBES, Rochester.

Much attention has been given to the dynamic state of bone and skeletal muscle electrolyte. Even though decreases in bone sodium and carbonate concentration and changes in muscle sodium and potassium concentration have been observed in animals with various types of extracellular acidosis, there are no reported studies of bone composition during diabetic acidosis. In this work observations were made of alterations in bone and skeletal muscle composition during alloxan-diabetic acidosis in adult rats.

A severe degree of hyperglycemia and acidosis was produced by the intravenous injection of alloxan. Each diabetic rat had a control animal which was treated identically except that alloxan was not injected. In some cases a second control, given no treatments, was also used. Since the exact age of each rat was known, it was possible to kill, coincidentally, diabetic and control rats of identical ages. Radiosodium, injected intra-abdominally before killing, was used to determine bone sodium exchangeability.

It was observed that on a fat-free-solids basis skeletal muscle water and potassium decreased while sodium remained the same. This is in agreement with the observations of others.

Observed values for total bone sodium and radiosodium content were corrected for "extracellular" fluid sodium and radiosodium by assuming that the entire water content of bone is freely permeable to the ions of extracellular fluid.

The average decrease observed in the "extra," or crystal, sodium concentration of bone amounted to approximately 6% of the total crystal sodium concentration. This change was of smaller magnitude than that observed by others using ammonium chloride dialysis in adult rats, even though the acidosis, in our animals, was of comparable duration and degree. Changes in crystal sodium concentration were not well correlated with changes in serum sodium concentration and pH or with changes in bone carbonate and water concentration. The crystal sodium exchangeability of bone also was observed to decrease by approximately 6%, indicating a loss of approximately 12% of the rapidly exchangeable crystal sodium.

Bone carbonate concentration was observed to decrease an average of 8%. Changes in bone carbonate content correlated well with the changes in pH but not with changes in serum carbon dioxide content or calculated pCO_2.

Observed decreases in bone potassium concentration were not significant.

Marked age variations in bone sodium, carbonate, calcium, and water content were observed in the untreated controls during a 30-day period. If age had not been taken into account in designing the experiment, changes in bone composition of a much different magnitude could have been observed.

Occurrence of Urinary Acid Mucopolysaccharides in the Hurler Syndrome. ANDREW E. LORINCZ* and ALBERT DORFMAN, Chicago.

The presence of abnormal amounts of a chemically unidentified substance in tissues of patients with the Hurler syndrome (lipochondrodystrophy, gargoylism) has been recognized. Brante (1951) isolated from tissues, obtained at autopsy, a polysaccharide thought to be similar to or identical with chondroitinsulfuric acid. Since acid mucopolysaccharides are not known to be synthesized in the parenchymal cells affected in this disease (e. g., liver, spleen, nervous tissue) it seemed possible that widespread deposition in tissues results from an increase in circulating mucopolysaccharides. Accordingly, an attempt was made to determine whether urine of patients with this disorder contains unusual amounts or types of mucopolysaccharides.

Application of appropriate methods for the isolation of acid mucopolysaccharides from urine resulted in the recovery of approximately 100 mg. of crude mucopolysaccharide per 24-hour collection from two afflicted children. These quantities are approximately 10 times that obtained from the urine of normal persons of comparable age and sex.

Further purification by slab electrophoresis of the crude mucopolysaccharide obtained from the persons with the Hurler syndrome permitted the isolation of two mucopolysaccharide peaks. The more rapidly migrating peak, which contained most of the material, was identified as chondroitinsulfuric acid B (β-heparin) on basis of analyses, resistance to testicular hyaluronidase, optical rotation, and antithrombic activity.

Preliminary studies on the more slowly migrating peak indicate that it is similar to heparin monosulfuric acid, previously isolated from liver by Jorpes and Gardell (1948).

These findings support the suggestion that the distortion of tissues in this inheritable disorder may be related to an abnormality of mucopolysaccharide metabolism.

Congenital Genetic Agranulocytosis. A. LEONARD LUHBY,* FRANCIS D. SPEER,* ROBERT LEE,* and ALLEN DAVID SHAPIRO,* New York Medical College. Introduced by EUGENE KAPLAN.

We have observed a severe agranulocytosis in a 3½-month-old infant who was known to have had

* By Invitation

this condition since the age of 6 weeks. His illness apparently began at the age of 2 weeks, with an umbilical stump cellulitis, and soon manifested itself as a severe agranulocytosis, which persisted without change until the child died with overwhelming infection, at the age of 7 months. We are of the opinion that this blood aberration probably represents a congenital genetic agranulocytosis due to an inborn error of development and maturation of the neutrophilic polymorphonuclear leukocyte series.

The significant clinical findings were as follows: Throughout the course only an occasional neutrophilic polymorphonuclear leukocyte was seen on smears taken almost daily. The total peripheral white cell count ranged from 4100 to 24,300 per cubic millimeter. Platelet and reticulocyte counts were within normal limits. There was a persistent mild normochromic anemia. The peripheral blood differential count contained essentially eosinophils, lymphocytes, monocytes, and an occasional plasma cell. Only the monocytes were moderately increased in total numbers. Examination of the bone marrow by needle aspiration revealed a cellular marrow with a striking reduction of the neutrophilic myeloid elements to 1.5% and the band and segmented polymorphonuclear cells to 1%. Plasma cells totalled 4%. The distribution of the remainder of the cellular elements of the marrow was normal. There was no increase of promyelocytes. Liver-function tests were normal. There was an unusually high level of α-, β-, and, especially, γ-globulin in the serum. Examination of the plasma for leukocyte antibodies by several techniques was uniformly negative. The course was marked by persistent fever and refractory skin abscesses. Smears of the latter showed the cellular reaction to be primarily monocytic and lymphoid with an occasional eosinophil. Cultures grew hemolytic Staphylococcus aureus, coagulase-positive. Despite antibiotics and surgical drainage, the patient died as a result of overwhelming staphylococcal pneumonia with multiple abscess formation.

The hematologic pattern and course appear to rule out agranulocytosis resulting from drug idiosyncrasy or infection. The condition did not resemble cyclic or periodic agranulocytosis, splenic neutropenia, or transient neonatal agranulocytosis, recently described from our laboratory. Absence of an abnormal cellular infiltration in any of the organs on postmortem examination points against a neoplastic or leukemic process.

It appears we are dealing with a rare type of agranulocytosis which was congenital. We feel that it has the features of an error of development on a genetic basis because of the void in the neutrophilic myeloid elements of the marrow.

Kostmann, in Sweden, recently described the occurrence of a condition apparently identical with that of our patient, in 14 children belonging to nine families. He presents a genetic analysis which appears to support the conclusion that the disease is primarily caused by a single recessive autosomal gene difference. There is no consanguinity between the parents of our patient. In none of the children of the siblings of the parents have we uncovered to date another instance of this disease.

Urinary Formiminoglutamic Acid, a Biochemical Test for Folic Acid Deficiency. A. Leonard Luhby* and Harry P. Broquist,* New York Medical College. Introduced by Eugene Kaplan.

The detection of a compound in urine, presumably identical with formiminoglutamic acid, was found useful as a new determinant of folic acid deficiency in humans. This may thus offer a specific biochemical means of identifying and studying this clinical deficiency state, which previously depended upon the demonstration of a megaloblastic anemia, refractory to cyanocobalamin (vitamin B_{12}) but responsive to folic acid.

Bakerman, Silverman, Daft, et al., reported that in folic acid-deficient rats the urine, after heat or alkali treatment, contained increased glutamic acid as measured by L. arabinosus microbiological assay. The increase was directly proportional to the granulocytopenia occurring in these rats and hence was presumably proportional to the degree of folic acid deficiency. There was a decrease in detectable glumatic acid following heat treatment of the urine of the normal rat as well as after folic acid therapy in the PGA-deficient animal. Tabor et al. demonstrated that the glutamic acid precursor in the urine of the deficient rat, which prior to heat treatment is not utilized by L. arabinosus, was formimino-L-glutamic acid and resulted from the incomplete degradation of L-histidine.

Extending these observations, we found a glutamic acid derivative in the urine of human subjects in certain acid-deficieny states, which is thus far microbiologically and chromatographically indistinguishable from formimino-L-glutamic acid.

The administration of folic acid antagonists to children with acute leukemia resulted in a progressive twofold to threefold increase of microbiologically detectable glutamic acid in heated urine specimens. Paper chromatography of these urines revealed the presence of a material which travelled at the same rate as synthetic formimino-L-glutamic acid. Heated urine of normal children and adults uniformly showed a fivefold to tenfold decrease in glutamic acid as compared with unheated urine. Prior to and following withdrawal of folic acid-antagonist therapy in leukemic children, as well as in those receiving other types of therapy, the urine exhibited the normal pattern.

* By Invitation

The urine of two patients with macrocytic anemia of pregnancy also showed microbiological properties characteristic of formiminoglutamic acid, which disappeared following folic acid therapy. The urine of several patients with pernicious anemia in relapse was "negative."

These observations will have the additional significance of demonstrating for the first time the existence of a biochemical abnormality in clinical folic acid-deficiency states dependent upon folic acid for formimino (—CH=NH) group transfer in amino acid metabolism, if isolation studies in progress prove the precursor compound in human urine to be formimino-L-glutamic acid.

The "Puff-Tusser," a Mechanical Device for Cough-like Inflation and Deflation of the Lungs. PAUL R. LURIE, Indiana.

Seriously ill patients who are unable to cough effectively often need mechanical assistance in removal of tenacious secretions from the lungs. Existing methods are not always satisfactory. The "Puff-tusser" was developed and is under study as a method which closely resembles the physiologic cough, yet requires no patient cooperation.

This device has an inspiratory phase, a phase resembling expiration against a closed glottis, and, finally, a phase of unimpeded expiration. Its components are (1) a reservoir of any breathable gas under sufficient pressure to pass a quick inspiratory jet through a face mask or endotracheal tube, (2) a reservoir of air connected to an inflatable bag enclosing the trunk, and (3) a system of valves and controls to effect the desired sequence of inflation and deflation in the two systems. For a brief controllable period inspiration (insufflation) is unopposed; then external counter-pressure is applied to the trunk. This prevents overexpansion of the lungs by counteracting expansion of the thorax and descent of the diaphragm. This period of rising intrapulmonary pressure with little change in volume substitutes for the normal phase of expiratory effort against a closed glottis. Then at controllable intervals the inspiratory force is brought abruptly to zero, and finally the external bag pressure is released.

As the forces at the operator's disposal are considerable and the possibility of doing harm exists, most of the study to date has been in dogs, with a few clinical trials in severely ill patients, where all other methods had failed. The report to be given is a description of the device and a presentation of the findings in animals, including simultaneous tracheal air flow and intrathoracic pressure recordings, as well as motion pictures of the lungs, tracheobronchial tree, and diaphragm during "cough," filmed through an x-ray image amplifier.

Clinical Problems of Hypothermia: Control of Ventricular Fibrillation. JAMES A. MANNING* and EARLE B. MAHONEY,* Rochester. Introduced by GILBERT B. FORBES.

The frequency of ventricular fibrillation occurring during cardiac procedures under hypothermic conditions has been a major risk factor that has restricted the clinical use of the technique.

The reversion of the arrhythmia to a regular sinus rhythm and the restoration of an effective cardiac "pump" with an adequate output is a much more difficult maneuver under hypothermic conditions than at normal temperatures. Electrical methods of defibrillation proved relatively ineffective in our hands, with various shocking currents and types of electrodes.

A technique was developed with use of potassium salts to stop the fibrillation by producing cardiac arrest, both ventricular and auricular, and by the venous infusion of hypertonic glucose solutions in a so-called "reactive" stage of cardiac function. This combination of agents stopped the arrhythmia and resulted in the extremely rapid restoration of an "effective" cardiac output in canine subjects.

The experimental data are presented, and our clinical experience with this defibrillation technique is reviewed. Possible mechanisms of action are considered.

We feel that the use of a potassium salt-glucose combination in the manner we have outlined is a most effective technique for the management of ventricular fibrillation occurring during cardiac surgery.

P^{32}-Labeled Isoflurophate (DFP^{32}), a Label for Leukocytes. A. M. MAUER,* J. W. ATHENS,* and HELEN ASHENBRUCKER,* Utah. Introduced by VINCENT KELLEY.

Measurement of leukocyte "survival time" has been attempted by labeling nucleic acids of leukocytes with C^{14} or P^{32}. These methods have proved impractical because of the lengthy, complex procedures involved in the isolation and counting of the nucleic acids and the large amount of blood required for an adequate sample. Furthermore, because of reutilization of the radioactive compound, interpretation of the curves obtained has been difficult.

Isoflurophate containing P^{32} (DFP^{32}) binds quickly and irreversibly to certain enzymes, and it has been found to label leukocytes in vivo. Following breakdown of the enzymes, this compound is released as diisopropylphosphate (DIP), which is promptly excreted without further utilization. These characteristics suggested the use of DFP^{32} in studying leukocyte physiology.

The DFP^{32} is injected either intravenously or intramuscularly. At appropriate intervals 20 cc.

* By Invitation

of blood is obtained. The red cells are removed by dextran sedimentation and lysolecithin. At the centrifuge speeds used, platelets are removed in the supernatant solutions. The resulting "button" of leukocytes is plated between two discs of scintillating plastic, and the radioactivity is measured in a scintillation counter.

Studies have been done in 16 normal subjects. Activity remains in the leukocytes for an average of 19 days, with a range of ±4 days. During the first two days a rapid fall of activity occurs, followed by a period of relatively constant activity until the 9th and 11th days, when the activity again declines until none remains at about the 19th day.

If amounts of DFP from 1.0 to 4.0 mg. are given by either the intravenous or the intramuscular route, the general shape of the curve is preserved and the end-point is unchanged.

Although the interpretation of this complex curve needs further clarification, the simplicity of the method and the reproducibility of results obtained in the normal subjects recommend the method for study of leukocyte physiology in normal and abnormal conditions.

Development of Serum Coagulation Factors in Infants. ARTHUR E. MCELFRESH,* Temple. Introduced by VICTOR C. VAUGHAN.

The multiple deficiencies of coagulation factors in the blood of infants offer a unique opportunity to study the interrelationships of these factors. Measurements have been made during the newborn period and for the first two months of life of prothrombin, proaccelerin, proconvertin, PTC, Stuart factor, and another un-named serum factor (Car) and of the total ability of infant's serum to correct the defect present in serum obtained following biscoumacetate (Tromexan) medication.

The results of such measurements and an analysis of the data will be presented. The data suggest that the factors in serum which are adsorbed by barium sulfate increase at similar rates and may be related in activity.

Blood Ammonia Levels in the Newborn. JOSEPH J. MCGOVERN,* WILLIAM V. MCDERMOTT JR.,* MARY H. MCGOVERN,* and MARY RUSSELL,* Harvard. Introduced by ALLAN M. BUTLER.

The concentration of ammonia in the blood was determined in parturient women at the time of delivery and in their infants immediately after birth, by a modification (McDermott, W. V. Jr., and Adams, R. D.: *J. Clin. Invest.* 33:1, 1954) of the microdiffusion technique of Conway (Conway, E. J.: Microdiffusion Analysis and Volumetric Error, Ed. 3, London, Crosby Lockwood & Son, Ltd., 1950). Blood was obtained from the antecubital vein of the mothers and the umbilical and femoral veins of the infants. In addition, a sample of cord blood was obtained from the placenta just after its delivery from the uterus. Further studies were carried out on samples of blood taken from the umbilical and femoral veins of infants at intervals during the first week of life. Comparisons were made of the ammonia levels in the umbilical vein before, during, and after exchange transfusions.

The results of the determinations revealed that (1) the ammonia level of the blood obtained from the mothers fell within the normal range of the peripheral blood ammonia level of adults; (2) the peripheral blood of newborns showed a significant elevation of blood ammonia immediately after birth, which may be related to (3) very high levels of blood ammonia obtained from the umbilical vein, and (4) the cord blood obtained from the placenta contains moderately high levels of ammonia, which tend to increase the longer the blood is permitted to stagnate before sampling occurs.

The data show that the peak of the blood ammonia levels appear immediately after birth and that level gradually declines during the first week of life. Inasmuch as blood in the portal vein normally contains exceedingly high levels of ammonia, it is probable that the communication between the portal and umbilical vein, which permits some blood to bypass the liver, contributes to the elevation of the ammonia level in the peripheral blood of the newborn. The return of the ammonia level to normal probably coincides in point of time with the reduction in patency of the ductus venosus.

Additional data were collected on the influence of storage on the ammonia level of citrated blood. The results confirm the work of others (Spear, P. W.; Sass, M., and Cincotti, J. J.: *J. Lab. & Clin. Med.* 48:702, 1956) that there is a roughly linear increase in ammonia concentration with time, at least in the first 10 days of storage. Serial determinations of blood ammonia before, during, and after exchange transfusions indicated that after exchange transfusion the infants' blood may contain greater or lesser concentrations of ammonia than were present before transfusion. There appeared to be a direct correlation between the post-transfusion ammonia level in the infant and the ammonia level in the blood used for transfusion. This would signify the advisability of using blood with low levels of ammonia, i. e., as fresh as possible, for exchange transfusions, particularly in infants in the first day of life, whose ammonia level is at its highest.

* By Invitation

Studies on the Behavior and Purification of the Serum Trypsin Inhibitor. FREDERIC C. MOLL, Washington.

The serum trypsin inhibitor is an α_1-globulin and can be partially purified by an ion-exchange resin, with a 50% yield and a twelvefold enrichment. This fraction is effective against trypsin but not against plasmin. It is heat-labile and inactivated by a pH below 5.

The behavior of the fraction has been studied in normal and disease states in humans and experimental animals to ascertain its function and mechanism of control. The administration of steroid hormones raises the blood level of the trypsin inhibitor in the normal animal, as does the development of inflammation. Paradoxically, if the blood level is already elevated through either of these mechanisms the level will be lowered by the other.

(normal bl. level + inflammation = inc bl. level) + cortisone = dec bl. level

(normal bl. level + cortisone = inc bl. level) + inflammation = dec bl. level

These findings probably represent a mechanism of increased utilization. The experimental studies and pertinent clinical observations will be presented, and the interrelationship with clinical inflammation and response to hormone therapy will be discussed.

Congenital Stenosis of the Aortic and Pulmonary Valvular Areas of the Heart, with Special Reference to Indications for Early Surgical Relief. ARTHUR J. MOSS,* FORREST H. ADAMS, BERNARD J. O'LOUGHLIN,* and WILLIAM P. LONGMIRE JR.,* U. C. L. A.

Extreme degrees of pulmonary valvular stenosis with an intact ventricular septum possess many features in common with severe stenotic lesions at or below the aortic valve. In either condition, a great burden is placed upon the respective ventricular chamber and the cardiac output tends to become low and fixed. The end-result is reduced systemic and coronary blood flow. Sudden death occurring with or without recognizable evidence of cardiac failure is not uncommon. The need for early recognition and prompt surgical relief in malformations of this type has, in the past, not been emphasized. The age or weight of the patient should in no way influence one's decision to perform the special diagnostic studies necessary for a definitive diagnosis. We have found that right heart catheterization and selective angiocardiography, even in very young infants, is far less hazardous than a policy of watchful waiting.

The case histories of three patients selected from our pediatric cardiovascular service are presented to illustrate the danger of delay and the benefit of early recognition and treatment. The first patient was a 15-month-old infant who was found by catheterization studies to have severe pulmonary valvular stenosis with an intact ventricular septum. Three days later she suddenly died in the ward while awaiting surgery. An emergency thoracotomy and pulmonary valvulotomy were of no avail. The second patient was a 10-year-old child with subaortic stenosis who died during cardiac catheterization. An emergency thoracotomy and aortic infundibulectomy performed in the catheterization laboratory failed to restore heart action. Necropsy confirmed the presence of severe subaortic stenosis and also revealed a pinpoint-sized osteum of the left coronary artery. The third patient was a deeply cyanotic one-year-old infant whose catheterization studies indicated an extreme degree of pulmonary valvular stenosis, the systolic pressure in the right ventricle being 192 mm. Hg. An emergency valvulotomy was performed on the same day, and the patient showed immediate improvement. Three months later she was asymptomatic, and there was no visible cyanosis.

When malformations of the type under consideration are suspected, we feel that definitive studies should be carried out if there are signs of cardiac enlargement as determined by roentgenographic and electrocardiographic studies or by exercise intolerance. If the presence of aortic or subaortic stenosis is confirmed, surgical relief should be accomplished despite the age or size of the patient. With pulmonary valvular stenosis, surgery should be performed if there is exercise intolerance or if the systolic pressure in the right ventricle is 77 mm. Hg or more. If there are indications of a diminishing cardiac output, emergency surgical relief is justified.

Effects of Alkylating Agents on Embryonic Development. M. L. MURPHY,* Sloan-Kettering. Introduced by SAMUEL Z. LEVINE.

Five alkylating agents which are widely used in clinical practice, mechlorethamine (nitrogen mustard, bis[β-choroethyl]methylamine HCl), chlorambucil (p[N,N-di-2-chloroethyl]aminophenylbutyric acid), TEM (2,4,6-triethylenimino-s-triazine), Thio-TEPA (N,N',N''-triethylene-phosphoramide), and busulfan (Myleran, 1,4-dimethanesulfonyloxybutane) were tested for comparative toxic and teratogenic activity on the 12-day rat embryo.

The dose of the chemical which appears to cause 100% resorption of the implantation sites on the 12th day of gestation appears to be in the range of the maternal L. D_{∞}. One-third to two-thirds of this dose produces teratogenic effects. The higher doses also give some inhibition of fetal weight and length and, in the mother, failure of normal weight gain and bone marrow depression.

* By Invitation

Fetuses surviving to 21 days showed a syndrome of gross abnormalities consisting of encephalocele, cleft palate, abnormal digits, short crooked tail, and edema.

At comparable fractions of the adult L. D_{50} dose the severity of the teratogenic effects varied with the drug used. Chlorambucil produced the most severe changes and busulfan the least. The explanation for these differences may be variation in the rate of absorption of the chemical from the injection site or passage through the placenta as well as specific effect on the embryo.

Studies on the Effects of Antibiotics on the Endotoxin (Somatic Antigen) of Enteric Bacteria. E. NETER, O. WESTPHAL,* E. A. GORZYNSKI,* and O. LÜDERITZ, Buffalo.

Enteric Gram-negative bacilli contain as somatic antigen or endotoxin a protein-lipid-polysaccharide complex. The lipopolysaccharide component is responsible for both toxicity and pyrogenicity, and the polysaccharide determines serologic specificity. Both the complex and the lipopolysaccharide become readily attached to erythrocytes. Upon addition of homologous bacterial antibodies these modified red blood cells are specifically agglutinated and lysed in the presence of complement. The present study was undertaken to determine the effects of antibiotics on these endotoxins, with particular reference to erythrocyte affinity and antigenicity.

Appropriate dilutions of antibiotics were mixed with crude or highly purified lipopolysaccharide antigens obtained from Escherichia coli, salmonellae, and Shigella sonnei. The mixtures were incubated at 37 C for 30 minutes and then used for modification of sheep erythrocytes. The methods used in hemagglutination and hemolysis tests have been described in detail previously (*Ann. New York Acad. Sc.* 66:141-156, 1956). Antigenicity was determined by means of the hemagglutination and hemolysis inhibition tests.

It was found that several antibiotics, notably the polypeptides polymyxin B and neomycin, inhibit enterobacterial hemagglutination and hemolysis. With use of purified S. sonnei lipopolysaccharide (10μg. per milliliter) as little as 10μg. per milliliter of neomycin or 1μg. per milliliter of polymyxin B inhibited these reactions completely. Humycin, a new antibiotic, was effective in amounts of 50μg. per milliliter. In contrast, as much as 10,000μg. per milliliter of bacitracin, another polypeptide antibiotic active against Gram-positive organisms, was required. Penicillin (1000 units per milliliter), erythromycin (500μg. per milliliter), oxytetracycline (Terramycin) (1000μg. per milliliter), novobiocin (500μg. per milliliter), as well as corticotropin (500μg. per milliliter) and various peptones were ineffective. It is significant that neither prior treatment of erythrocytes with neomycin and polymyxin B followed by modification with antigen nor treatment of previously modified red blood cells with the antibiotics produced the same effect. Hemagglutination and hemolysis inhibition tests revealed that the antigenicity of the endotoxin is decreased but not abolished by these antibiotics. Therefore, the inhibition of the reactions is due to interference with attachment of endotoxin to erythrocytes and/or alteration of antigenicity rather than due to action on antibody, complement, or the agglutination and lysis reactions per se. That neomycin and polymyxin B do not affect all bacterial hemagglutination and hemolysis reactions is evident from the observation that these antibiotics were ineffective in the system employing non-species-specific streptococcal antigen, obtained from Dr. Rantz.

The above results will be discussed with reference to the questions whether (1) neomycin and polymyxin B also affect toxicity and pyrogenicity of the endotoxin and thus act as antitoxic and antipyretic agents, (2) the interaction of endotoxin and antibiotics represents the first step in antibacterial action of the latter, (3) the antibiotics after interaction with the endotoxin retain their efficacy as antibacterial agents, e. g., in the intestinal tract, and (4) the endotoxin of antibiotic-resistant enteric bacteria is similarly affected by the antibiotics as that obtained from susceptible strains.

Family and Community Problems of Hemophilia. DENNIS D. PATTON,* GRAYDON N. FUNKE,* and NATHAN J. SMITH, California.

This study was undertaken to identify problems more clearly in family and community relationships of the hemophilia patient. The manner in which the hemophilia patient meets the problems of his abnormal relationship to his environment was studied.

Questionnaires were sent to 220 families of patients with hemophilia of known type (A and B). One hundred eighteen questionnaires were returned, of which one hundred nine were suitable for analyses.

Economic demands of the disease were evaluated in relation to type, age of patient, and family income. The ability of older patients to become economically self-sufficient was apparent, and interesting vocational guidance data have been obtained. In general, families with young patients with hemophilia cannot meet the economic demands of the disease without financial assistance.

Several facets of school problems were studied. Most parents do not wish their hemophiliac children to attend "special schools." It was of interest to find that most hemophiliac students maintain normal academic achievement patterns in school.

* By Invitation

More than half of the hemophilia patients studied failed to admit to a family history of hemophilia. The sibling and parent relationships, discipline patterns, and residence as influenced by the disease were evaluated from the questionnaire data.

Information as to the age and mode of diagnosis and eugenic aspects of this disease will be discussed.

A Micro Method for Determining Butyl Alcohol-Extractable Iodine in Serum. D. E. Pickering and N. Kontaxis,* Oregon.

There has been developed a method for determining serum butyl-extractable iodine, employing no distillation technique and adapted to the standard Beckman D. U. Spectrophotometer. Serum (0.25 to 0.5 cc.), upon acidification, is extracted with normal butyl alcohol. The extract is then appropriately washed with Blau's alkaline reagent for separation of M. I. T., D. I. T., and iodide. The thyroxine-containing normal butyl alcohol is then dried, and the residue is ashed by alkaline fusion. The ashed specimen is solubilized, and its iodide content is determined by its catalytic effect on the cericarsenious oxidation-reduction reaction.

The concentrations of reactants, the temperature, and the period of reaction have been adjusted to exaggerate to a maximally sensitive first-order reaction the catalytic effect of iodide.

Adaptation of this method to the Beckman D. U. Spectrophotometer has been achieved by employing the inhibitory effect of silver ion on iodide catalysis of this reaction.

Data demonstrating the various facets of this procedure, recoveries, and its potential clinical and research usefulness will be presented.

Observations on the Toxic Effect of Ozone on Newly Hatched Chicks. J. J. Quilligan Jr., R. D. Boche,* P. Kotin,* and H. Falk,* College of Medical Evangelists and University of Southern California.

The problem of air pollution in our large urban communities is becoming as important as the problems of water pollution were 50 years ago. Since the composition of the polluted air varies with the community, this compounds the difficulties. In Los Angeles, gasoline exhaust, industrial smoke wastes, and incinerators are the major causes of the pollution. The interaction of these components, in the presence of sunlight, results in the formation of ozone, which in turn causes other reactions, giving a very complex product. The harmful significance of the various components of naturally occurring air pollutants will require considerable investigative effort before they are properly defined. Nevertheless, the effects of some of these have been categorized and ozone is one of them.

Ozone has been shown to be definitely detrimental to certain types of plant tissue. In addition, considerable information on the harmful effects of ozone on various animal species is available. However, there are no data on the use of the young chick in demonstrating ozone toxicity.

During the course of investigating the influence of synthetic air pollutants on influenza virus infections of laboratory animals, it was observed that chicks react to the effects of low concentrations of ozone with a hemorrhagic pneumonitis. The use of this animal as a means of demonstrating ozone toxicity has given data suggesting that its susceptibility to the lethal effects of ozone is greater than that of other commonly used laboratory animals.

Treatment of Acute Respiratory Distress in Newborn Infants of Diabetic and "Prediabetic" Mothers. Helen S. Reardon, Sasha Field,* Luis Vega,* Elsie Carrington,* James Arey, and M. L. Baumann,* Temple.

A group of 55 newborn infants were observed in this study. Forty-two were infants of diabetic mothers, and thirteen were infants of mothers with laboratory evidence of abnormal carbohydrate metabolism only during pregnancy, i. e., "prediabetic" mothers. These infants, all of whom were considered to be in respiratory distress, exhibited cyanosis, change in respiratory rate and/or rhythm, changes in muscle tone and reflexes, and usually hepatomegaly and edema. All infants were given general supportive treatment, consisting of controlled environmental temperature and humidity and O_2 therapy. Starting with umbilical cord samples and for the first 3 days of life the infants had arterial blood chemical studies consisting of pH, CO_2, total base, K, Cl, protein, and glucose. Values for plasma pCO_2 and "R" were calculated.

Severe respiratory acidosis (increased umbilical artery pCO_2) existed at the time of birth in the infants who subsequently developed symptoms of respiratory distress. At 4 hours of age, in addition to respiratory acidosis there was also a metabolic acidosis, as indicated by a decreased plasma CO_2 content, but this was not worse than in normal infants at the same age. The blood glucose concentration at 2 and 4 hours of age was decreased significantly as compared with that of well infants.

Eighteen infants examined in a period from 1953 to 1955 were given 10% glucose water either parenterally or by mouth or received no fluids. Six of these infants died between 11 and 50 hours of age, and all of them had pulmonary hyaline membranes at autopsy. Thirty-seven infants in the period from 1951 to 1957 received 30 ml. per lb. per day of 5% glucose and 0.45% saline. Therapy was usually initiated within 2 to 12 hours of birth and was associated with marked clinical improve-

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ment in all but one patient and a return of arterial pCO_2 to normal within 24 hours. Blood glucose required a longer period for stabilization. Three of these infants died, one with pulmonary hyaline membrane at 4 hours, one with hypoglycemia and infection at 7 days, and one at 21 days, possibly as a result of sepsis. The difference in the death rate associated with pulmonary hyaline membranes in the two groups is significant at the 1% level.

The lowering of the arterial pCO_2 indicates an improvement of aeration of the blood. The mechanism by which this is produced is unknown but may be concerned with either the lungs or the cardiovascular system.

The Absorption of I^{131}-Labeled Fat and Fatty Acid in Cystic Fibrosis of the Pancreas. KEITH REEMTSMA,* PAUL A. DI SANT'AGNESE, JAMES R. MALM,* and HAROLD G. BARKER,* Columbia.

The presence of normal pancreatic function and of variations in the degree of pancreatic insufficiency in cystic fibrosis of the pancreas has been reported recently. These observations have aroused interest in the mechanism of malabsorption in this condition, and the development of isotope-tagged fat and fatty acid tests provides a new method of evaluating pancreatic function.

The test involves the oral administration, on separate occasions, of I^{131}-labeled olive oil (neutral fat) and its principal fatty acid, oleic acid. Intestinal absorption is estimated from serum radioactivity, and the results are expressed in percentage of total administered dose in the serum.

Normal values were established in 27 adults, and studies on seven children without gastrointestinal disease followed the same pattern seen in normal adults. The normal mean value at the peak of serum activity is 14.7% following ingestion of neutral fat and 8.0% following ingestion of fatty acid.

Studies on seven patients with established cystic fibrosis of the pancreas demonstrated marked impairment of neutral fat absorption and moderate impairment of fatty acid absorption. The median values at the peak of serum activity were less than 1% with neutral fat and 2.7% with fatty acid. This pattern was seen in two patients with cystic fibrosis of the pancreas who demonstrated normal tryptic activity in the duodenal aspirate.

These results are compared with observations on six adults with pancreatic deficiency due to resection, extensive fibrosis, or tumor of the pancreas. In this group fatty acid absorption is normal despite marked depression of neutral fat absorption.

The difference in behavior between these two groups of patients supports the opinion that cystic fibrosis of the pancreas is a generalized disease and that other mechanisms in addition to pancreatic deficiency are involved in the malabsorption in this disease.

Electroencephalographic Findings Associated with Cyanotic and Acyanotic Congenital Heart Disease. SAUL J. ROBINSON* and EDWARD E. SHEV,* San Francisco. Introduced by SYDNEY GELLIS.

In many cases of congenital heart disease with cyanosis hypoxia or even anoxia apparently exists, and to our knowledge a study of the electroencephalographic changes in such cases has not been reported. It was felt that congenital heart disease would represent a group of conditions where it could be determined if electroencephalographic changes occurred and whether the presence of cyanosis in any way affected the electroencephalogram.

Twenty-eight patients were examined whose cardiac disease entities were divided into two groups, depending on whether cyanosis was or was not present. The ages ranged from 4 days to 18 years, with an equal distribution of males and females. There were 15 cyanotic and 13 acyanotic cases. These children, in addition to the history and physical examination, had x-rays, electrocardiograms, blood counts, urinalyses, and, in some instances, cardiac catheterizations and oxygen saturation values, although the relationship of the oxygen saturation to the electroencephalographic findings was not evaluated. In addition, complete neurological examinations were performed in those cases where an abnormal neurological condition was suspected. Neurological entities were present in eight children, consisting of seven cases of epilepsy and one case of Mongolism, with four children in each group with neurological diagnoses. More than one electroencephalogram was performed on several of these children, but with consistent findings, and so that only one record is reported per child. The methods of obtaining the electroencephalograms followed a standard procedure.

Results.—Of the 28 cases studied there were 16 with electroencephalograms which were considered by E. S. to be abnormal, and 12 were considered to be within normal limits. In the group with cyanotic congenital heart disease, consisting of 15 children, there were 13 electroencephalograms considered to be abnormal and only two, normal. In the acyanotic group, consisting of 13 children, there were 3 abnormal electroencephalograms and 10 normal tracings. Expressed in percentages, the cyanotic group had 86% abnormal electroencephalograms, and the acyanotic group had 30% abnormal tracings. There thus seems to be a consistent relationship between the presence of cyanosis and the

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abnormality of the electroencephalographic tracing, particularly if one excludes those children with obvious neurological conditions.

Conclusion.—There appears to be a definite relationship between the presence of cyanosis in congenital heart disease and the abnormality of the electroencephalogram. This represents a preliminary report on a comparatively small group of children, and the study is being continued.

Persistent, Probably Congenital, Hypokalemic Metabolic Alkalosis with Hyaline Degeneration of Renal Tubules and Normal Urinary Aldosterone. P. ROSENBAUM * and M. HUGHES,* Louisiana. Introduced by N. K. ORDWAY.

Persistent, probably congenital, hypokalemic metabolic alkalosis in an infant showing hyaline degeneration of renal tubules, and in whom urinary aldosterone excretion was normal, is the subject of this report. Diarrhea, described as a cardinal feature in previous publications delineating this biochemical aberration, was sporadic in this infant and apparently did not account for the observed clinical and chemical features. Normal urinary excretion of aldosterone, 17-ketosteroids, and chemical cortins, associated with histologically normal adrenals at autopsy, appears to exclude adrenal dysfunction as a primary etiologic factor.

The patient, a Negro boy, was seen initially at 2 months of age because of diarrhea, dehydration, and poor weight gain. The clinical course was characterized by repeated bouts of dehydration in the presence of continuing good urinary output and pitting edema of the lower extremities. These episodes showed no consistent relationship to excessive gastrointestinal losses. Severe anorexia and repeated infections became prominent. Hypokalemic alkalosis persisted despite the addition of as much as 8 gm. of potassium chloride to a variety of daily formulae. The infant died at the age of 7½ months, weighing less than at birth.

Laboratory Findings.—Except in rare instances, blood pH remained elevated, reaching a maximum of 7.69. Serum potassium and chloride concentrations were usually low, and serum sodium ranged from normal to low. In general, an inverse relationship between serum potassium and blood pH was demonstrated. Blood urea nitrogen was not elevated, and total serum proteins were at the lower range of normal.

Urinary studies showed inability to concentrate above 1.015. The pH ranged from alkaline to slightly acid. Tests for albumin and sugar were negative. Cultures were negative except terminally. No abnormal amino acid excretion was demonstrated by paper chromatography. Two 24-hour urinary aldosterone excretions were normal. On two occasions most of the administered KCl was excreted in the urine, potassium concentrations as high as 90 mEq. per liter being recorded.

Stool pH was generally alkaline, but an occasional acid reaction was noted. Sweat sodium was 6 mEq. per liter; potassium, 14 mEq. per liter.

Autopsy.—Autopsy studies disclosed hyaline degeneration of renal tubules, normal glomeruli and arterioles, and no evidence of renal infection. Adrenals were normal. The liver showed marked fatty changes.

Circulatory Changes Associated with Pulmonary Valvular Obstruction During Cardiac Catheterization. ABRAHAM M. RUDOLPH * and MILTON H. PAUL,* Harvard. Introduced by CHARLES A. JANEWAY.

A serious and not infrequent complication of cardiac catheterization has been encountered in eight patients with severe pulmonary stenosis. During cardiac catheterization in 31 children with pulmonary valvular stenosis with intact ventricular septum, hemodynamic changes indicating obstruction of the pulmonary valve by the exploring catheter were observed in 8 (25%) of the patients studied. In six instances acute clinical evidence of the obstruction was observed—apprehension, increasing cyanosis, myoclonic contractions, and, in one instance, hemiparesis of several days' duration.

Nine of the thirty-one patients showed arterial oxygen unsaturation, and the eight patients with signs of valve obstruction fell in this group. The resting pulmonary blood flows were considerably lower (2.1 liters per minute per square meter) than in the nonobstructed group (4.8 liters per minute per square meter), and the pulmonary valve size, as calculated by the Gorlin formula, was less than 0.15 sq. cm. in all patients with evidence of obstruction.

During the presence of the catheter in the pulmonary valve, the pulmonary blood flow, already reduced, was decreased even further to very low levels. There was a marked reduction in mixed venous and in arterial oxygen saturation; this decreased arterial saturation was associated with an increase, or relative increase, of the right-to-left shunt through a patent foramen ovale.

The inability of the right ventricle to meet the increased demands placed on it by the presence of the catheter in the pulmonary valve will be discussed in relation to the pressure gradients required to maintain an adequate pulmonary blood flow through various valve areas.

Idiopathic Obstructive Jaundice in Early Infancy (Neonatal Hepatitis). A. SASS-KORTSAK, D. H. BOWDEN,* O. V. SIREK,* and M. CHERNIAK,* Toronto.

This report is based on observations collected on 25 cases of obstructive type of jaundice in early infancy. Congenital extrahepatic biliary atresia,

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galactosemia, preexisting hemolytic disease of the newborn, sepsis, and syphilis as possible causes for the obstructive jaundice were excluded.

In two cases of this idiopathic group the diagnosis of fibrocystic disease was made based on pancreatic enzyme and sweat-electrolyte studies. It is conceivable that in these two cases the obstructive jaundice was a manifestation of mucoviscidosis. Liver-function studies showed the presence of uncomplicated biliary obstruction without evidence of parenchymal damage. Clinical and laboratory features will be demonstrated leading to the conclusion that fibrocystic disease should also be considered as the underlying pathology in cases of otherwise unexplained infantile obstructive jaundice.

On the basis of detailed serial liver-function studies the remaining 23 cases could be subdivided into two groups.

The larger group had abnormal tests indicating biliary obstruction only and had no laboratory sign of parenchymal damage even after repeated observations. From a clinical, laboratory, and pathological point of view these cases are similar to cases of "intrahepatic obstructive jaundice" or "cholangiolytic hepatitis" in adults. A high percentage of these cases developed cirrhosis which resembled biliary rather than the portal type of cirrhosis from a laboratory and clinical point of view.

A smaller but distinct group of patients exhibited definite laboratory signs of abnormal parenchymal function in addition to the signs of biliary obstruction. These cases from both a clinical and a laboratory point of view resembled cases of viral hepatitis in adults or older children. All patients in this group made a complete recovery from the point of view of liver function. One developed marked mental retardation.

There was one family in each group with multiple incidence in siblings of the same type of condition.

It has been suggested by others that this condition is a type of hepatitis and is probably due to a viral agent. According to our findings it seems that at least two types of this condition can be distinguished, presenting with different laboratory findings. It may be that these two types are variants of the same disease. Recently, however, in addition to the virus of serum hepatitis, another viral agent, that of cytomegalic inclusion disease, has also been incriminated as an etiological factor. It is therefore, conceivable that different etiological factors may lead to variation of laboratory manifestations of the disease.

The Use of a Soybean Phosphatide as a Platelet Substitute. IRVING SCHULMAN, ZINET CURRIMBHOY,* ELEANOR FORT,* MARION E. ERLANDSON,* JULIAN B. SCHORR,* and CARL H. SMITH, Cornell.

Bleeding resulting from thrombocytopenia constitutes the most frequent hemorrhagic disorder encountered in clinical practice. Yet of all the coagulation factors present in blood the platelets are the most difficult to obtain and preserve in a physiologically active state. Apart from clinical use a source of platelets of constant and reproducible activity is also required for laboratory diagnosis of those coagulation disorders characterized by impaired thromboplastin formation (i. e., the various hemophilias). Problems in preparation of platelet suspensions have presented a major difficulty with the most sensitive test now available, the thromboplastin generation test.

It has been known for several years that the platelet thromboplastic factor is a lipid, and recently many phosphatides of animal and plant origin (human brain, red cells, soybeans, cotton seeds) have beeen successfully substituted for platelets in various in vitro tests. The present report concerns the in vitro and in vivo use of a soybean phosphatide as a platelet substitute.

The material used is the alcohol-insoluble fraction of soybean phosphatide and contains approximately two-thirds inositol, one-third ethanolamine, and a small amount of choline. On in vitro testing the soybean material has been found capable of substituting completely for platelets in the thromboplastin generation test. At the same time, the material possesses no AHG, PTC, or PTA activity and thus permits accurate diagnosis of the various hemophilias. In the diagnosis of mild defects the test becomes more sensitive with the phosphatide than with platelets. The soybean material also reduces the recalcification time and completely corrects the abnormal prothrombin consumption of plasma made thrombocytopenic by centrifugation and of plasma from thrombocytopenic patients. The abnormal prothrombin consumption in thrombocytopenic whole blood is likewise corrected, and clot retraction is improved.

The soybean phosphatide is active over a wide range of concentrations and is stable on freezing, boiling, and autoclaving. Large excesses do not produce hypercoagulability but are actually inhibitory.

Animal studies have disclosed no toxicity following intravenous injection of large doses of the material. Studies on the effects of intravenous administration of the soybean phosphatide to thrombocytopenic human subjects are in progress and will be reported.

* By Invitation

Determination of pH, CO_2 Content, O_2 Saturation, and Lactate in the Blood of the Newborn Infants of Diabetic Mothers. S. SEGAL,* J. M. SUTHERLAND,* J. F. LUCEY,* J. E. DROR-BAUGH,* R. B. CHERRY,* and D. Y. HSIA, Harvard.

In 1954, Lowrey et al. (Lowrey, G. H.; Graham, B. D., and Tsao, M. U.: *Pediatrics* 13:527-535, 1954) reported the finding of uncompensated acidosis in infants less than 24 hours old whose mothers had diabetes mellitus. Analyses of "arterialized" capillary blood indicated an elevated pCO_2 at this early postnatal age. It was to gain further data relative to the problem of acid-base balance in such infants that the present study was undertaken. The data to be presented (pH, CO_2 content, calculated pCO_2, O_2% saturation, and lactates) were obtained from a group of 45 infants whose mothers had diabetes and had been under the continuous care of the Joslin Clinic. Thirty-three of these infants were classified as "normal"; eight showed clinical or pathological evidence of "hyaline membrane syndrome" (four deaths); one had congenital heart disease; one, erythroblastosis fetalis, and two died with no anatomical cause being found at autopsy. The majority of the data to be presented were obtained from cord blood except in the case of lactates and pH. The one-hour and four-hour determinations were made on "arterialized" capillary heel blood samples.

Since all these determinations were not obtained on all infants in the series, the averages in the following Tabulation are derived from varying numbers of determinations as indicated.

	pH	CO_2 Content, mEq./L.	pCO_2, Mm. Hg	O_2, % Satura.	Lactate, Mg./100 Cc.
Cord artery	7.09(21) *	25.4(13)	82(13)	14(22)	25(25)
Cord vein	7.15(29)	23.2(17)	68(17)	37(25)	20(27)
One hour	7.26 (6)				18(17)
Four hours	7.27(8)				12(16)

* Figures in brackets indicate the number of determinations.

The data obtained from this group of infants of diabetic mothers indicate an acidosis at the moment of birth, with an elevation of both carbon dioxide tension and blood lactate level. In addition, the mean oxygen per cent saturation appears to be low. The findings suggest the possibility that an acidosis frequently exists at birth as well as at the postnatal ages observed by Lowrey et al. The suggestion that the placentas of these infants are unable to maintain normal function with respect to gas exchange between mother and fetus will be discussed.

* By Invitation

Inquiries into the Etiology of Acute Bronchiolitis in Infants: II. Koch's Postulates. SARAH H. WOOD SELL * and JOHN L. SHAPIRO,* Vanderbilt. Introduced by AMOS CHRISTIE.

Attempts to fulfill Koch's postulates with material obtained by nasopharyngeal swabs from three babies critically ill with acute bronchiolitis are here reported. Hemophilus influenzae, Types a and e, were the predominating organisms grown on cultures, and these were lyophilized. Bacteria-free nasopharyngeal filtrates which would contain any virus present were frozen.

Method.—Suckling mice, one day old, were inoculated intranasally. Group I received filtrate alone; Group II, filtrate plus pure cultures of H. influenzae isolated from the patient; Group III, H. influenzae alone. At the end of three to four days, the mice were killed. The lungs were cultured on Levinthal's agar and fixed in Bouin's solution. The microscopic sections were examined without identification except coded labels.

These experiments were performed three times on material from each patient.

Results of Examinations of Mice Lungs

	Sections	Cultures
Group I	No significant pneumonitis	No H. influenzae
Group II	Peribronchiolar pneumonitis, interstitial in early lesions but later confluent	H. influenzae recovered
Group III	Same as Group II	H. influenzae recovered
Controls		
No inoculum (normals)	No pneumonitis	No H. influenzae
Filtrate from patients not ill with respiratory infection	Heated: no pneumonitis	No H. influenzae
	Unheated: no pneumonitis	No H. influenzae
Saline blanks of culture materials	No pneumonitis	No H. influenza

Conclusion.—Koch's postulates have been fulfilled by H. influenzae recovered from the nasopharynx of patients with acute bronchiolitis in three controlled experiments.

Hydration Therapy in Sickle-Cell Anemia. J. RANDOLPH SHARPSTEEN JR.,* Temple. Introduced by ROBERT H. HIGH.

Hydration therapy is based on the theory that sickling is primarily an osmotic phenomenon and that the crisis is initially due to sequestration of the affected red blood cells. Water, when taken into the red cell will cause reexpansion of the sickled red blood cell and allow recirculation of the previously sickled red cell.

Over a four-and-one-half-year period 21 patients with sickle-cell anemia were studied at St. Christopher's Hospital for Children. There were 88 admissions for crisis during this period. Eighteen of the twenty-one patients had sickle-cell anemia

proven by electrophoresis. The remaining three had clinical courses typical of sickle-cell anemia. Oral hydration was used for approximately three and one-half years. The first years of the study was used as one control period. Transfusion and hydration therapy were used several times on each patient for comparison.

There was no significant reduction in the number of admissions during the hydration period as compared with the control period. There was no reduction in the number of crises. There was however, a shorter time between admissions following transfusion therapy than following hydration therapy. There was also a significant decrease in the patient's own hemoglobin following transfusion and an increase following hydration.

It is my feeling that hydration therapy is efficacious if used early enough in the crisis, i.e., before active hemolysis has occurred. However, no correlation between onset of the crisis, e.g., by measurements of serum bilirubin, reticulocyte count, per cent of nucleated red cells, red blood cell counts, or fall in the hemoglobin levels from patient's previous average, and efficacy of hydration therapy could be made on the present study.

Mongolism: Grading and Intercorrelation of Certain Measurements. THOMAS H. SHEPARD II,* ROBERT MCGREAL,* and SHERYL HAMMER,* Washington. Introduced by ROBERT ALDRICH.

A new method for analysis of clinical material has been used. Forty selected measurements from 60 Mongoloid children were recorded on IBM cards and 800 intercorrelation coefficients were quickly obtained by use of an IBM Type 650 magnetic-drum data-processing machine. In many cases it was necessary first to express the measures in percentiles. The heights, for instance, when compared to a normal percentile chart did not overlap to any extent, although the standard deviation was approximately the same.

The total degree of Mongolism was recorded as a function of height percentile, I. Q., cephalic index, hand involvement hyperextensibility, and eye involvement. The total degree of Mongolism correlated fairly well with the above criteria.

Correlations of Total Degree of Mongolism with Various Factors

	r	P
I. Q. percentile	+0.6483	0.001
Height percentile	−0.5480	0.001
Hyperextensibility	+0.4911	0.001
Cephalic index	+0.4277	0.001
Hand involvement	+0.3922	0.001
Eye involvement	+0.3151	0.01

A number of unexpected correlations with total degree of Mongolism were found. The span, pubic height, and weight percentiles (0.6483, −0.6038, −0.5695) correlated as well as the criteria. The percentage of polymorphonuclear leukocytes also correlated (−0.3542, P=0.01).

The congenital anomalies in this group were studied in a similar way, and some interesting correlations were obtained between type of anomaly and degree of pigmentation. (Mesodermal anomalies and degree of pigmentation, −0.3301, P=0.01.)

This work has been undertaken in an attempt to apply the technique of factor analysis to the correlation matrices obtained from this and a normal group. Through this it is hoped that certain differing central factors can be isolated and shown to represent abnormal genes and/or enzyme systems.

A Hemolytic State Associated with an Acute Pneumococcal Infection in Rabbits: Preliminary Report. CLARE N. SHUMWAY JR.* and GERALD MILLER, Rochester.

"Dermal pneumonia" is an acute inflammatory process produced in rabbits by the intradermal injection of living pneumococci. Jacox and Feldmahn noted that some rabbits thus infected became anemic. In contrast to the large body of information concerning the anemia of chronic infection, few data are available concerning the anemia which frequently accompanies acute bacterial infections in young human subjects. In view of this a study was undertaken to define the cause of the anemia experimentally produced in rabbits.

"Dermal pneumonia" due to Type I Pneumococcus was produced in 11 male white rabbits weighing between 2.1 and 3.9 kg. Twenty-four to forty-eight hours after infection the animals were severely ill; three died in the early phase of the illness. Data obtained during this period allow no conclusions regarding activation of a hemolytic process. Three to seven days after the onset of the infection six of the surviving animals were noted to be anemic. The anemia was associated with spherocytosis and increased erythrocyte osmotic fragility.

Some of the animals subjected to sham infections and comparable amounts of blood loss developed moderate nonspherocytic anemia and reticulocytosis. Therefore, conclusive statements cannot yet be made regarding the occurrence of hemolytic anemia in the infected animals. However, the occurrence of spherocytosis during the illness and its disappearance during recovery provide firm evidence of red cell damage accompanying the infection.

The procedure described provides another method for producing spherocytosis in vivo; the experimental model may be analogous to the anemia associated with some acute infections of childhood.

* By Invitation

Prevention of Rheumatic Fever: Effect of Prophylaxis in a High Streptococcal Disease Area upon Subsequent Recurrences of Rheumatic Fever. ALAN C. SIEGEL,* ELOISE JOHNSON,* and JOHN A. BIGLER, Northwestern.

Numerous reports have demonstrated the increased susceptibility of rheumatic subjects to recurrences of rheumatic fever, compared with initial attack susceptibility in normal subjects. The present study evaluates three methods of chemoprophylaxis given over a two-year period to 135 rheumatic children in order to observe the over-all attack rate for rheumatic fever recurrences, the relationship between streptococcal disease rates and recurrences of rheumatic fever, and changes in cardiac status in relation to freedom from or presence of streptococcal disease.

All children presented a definite recent history of acute rheumatic fever. They were placed into three prophylaxis groups according to a random method of selection. Group I (53 children) received sulfadiazine, 1.0 gm. per day by mouth in two divided doses, for the first 12 months of the study, and phenoxymethyl penicillin (penicillin V), 200,000 units (1 tablet) daily, for the second 12 months. Group II (41 children) received a single injection of benzathine penicillin, 1,200,000 units, every four weeks. Group III (41 children) received a single injection of benzathine penicillin, 1,200,000 units, every eight weeks.

All children were seen at four-week intervals, and throat cultures were obtained as well as blood samples. All β-hemolytic streptococci were isolated and grouped and typed with specific streptococcal antiserum. Antistreptolysin O determinations were performed at least bimonthly.

Seven recurrences of acute rheumatic fever, including two cases of chorea, occurred in the 135 children (2.5% per year), over the two-year period. All developed in children who had experienced related streptococcal infections. Three occurred in the 53 children on oral medication; one, in the 41 children receiving benzathine penicillin every four weeks, and three, in the 41 children receiving benzathine penicillin every eight weeks.

There were 55 streptococcal infections ascertained by either significant antibody titer rises and/or persistent positive cultures for Group A streptococci. Since these children were followed a total of 2664 patient-months, this represents a streptococcal disease rate of 2/100 patient-months in 135 children on prophylaxis. The disease relationships according to specific prophylaxis regimens will be discussed.

Group III affords the opportunity to relate maximal antibody formation, delayed eradication of the organism, and subsequent recurrences of rheumatic fever, and will be discussed.

* By Invitation

Salmonella Osteomyelitis and Abnormal Hemoglobin Disease. HENRY K. SILVER, JIMMY L. SIMON,* and DAVID H. CLEMENT,* Yale and Colorado.

Osteomyelitis as a complication of Salmonella infections is comparatively rare, occurring in less than 1% of all cases. Up to the present time 24 cases of Salmonella osteomyelitis and abnormal hemoglobin disease have been reported. In all of those in which details were given the patients were said to have been Negro and to have had sickle-cell disease. We wish to present the 25th and 26th cases of Salmonella osteomyelitis and abnormal hemoglobin disease; the first in an 11-year-old Caucasian boy with combined sickle cell-Mediterranean anemia and the second in a 3-year-old Negro with hemoglobin C-hemoglobin D disease. To our knowledge, this is the first time Salmonella osteomyelitis has been reported in a patient with mixed heterozygous hemoglobin disease (the first case) and the first time it has been observed in a hemoglobinopathy without sickling (the second case).

The increased incidence of Salmonella osteomyelitis in patients with abnormal hemoglobin disease has been explained in several ways. One explanation may be that the organisms lodge in the bone marrow following Salmonella septicemia and remain in a latent parasitic state for many weeks or months. In most cases the natural defenses of the body eventually destroy these organisms, but when certain abnormal hemoglobin diseases are present local factors such as infarction, thrombosis, and necrosis (which may be part of the primary blood disease) would enhance the possibility that the secondary infection would occur. Another factor operating to cause an increased incidence of Salmonella osteomyelitis in primary hemoglobinopathies may be due to the fact that the organism is more likely to localize in tissues with a poor vascular supply, so that humoral antibodies and administered antibacterial agents will not have an opportunity to counteract the infection.

A Reassessment of the Processing of Infant Formulas. HENRY K. SILVER and MILDRED D. FOUSEK,* Yale and Colorado.

Each year the mothers of approximately 3,000,000 infants spend from one-half to one hour daily for several months preparing and processing infant formula, nursing bottles, and nipples. In most instances physicians recommend making a new supply of formula each day and disinfecting by either the "terminal heating" or "aseptic" method. The "terminal heating" method consists of measuring and mixing the various ingredients in clean but not sterilized equipment and then heating the assembled formula units for 25 minutes in boiling water. With the "aseptic" method the equipment and mix-mix-

ture are boiled separately, and the milk is then added to the clean bottles with use of aseptic technique.

Although there is a comparatively extensive literature regarding the methods of processing of infant formulas, almost all of it concerns the preparation of such formulas for use in hospital nurseries. None of the reports discuss the length of time that formulas may be stored before using nor the various precautions that must be followed by mothers in order to insure safe formulas for their infants.

We have recently reassessed the methods of formula-making and heating as practiced by mothers. Tests were made of the efficacy of the two standard methods of heat treatment on uninoculated regular formulas and on formulas inoculated with substantial numbers of several test organisms, including Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes (β-hemolytic), Hemophilus influenzae, Pneumococcus, and Bacillus subtilis. The effect of preliminary washing of equipment, storage of heated formulas for varying periods of time, and of incubation at room temperature and at 98.6 F was also determined.

Summary and Conclusions

1. The "terminal" method of heating is the safest, most convenient, and most rapid means of producing artificial milk feeding for infants. However, its use should be accompanied by thorough cleaning of equipment, scrupulous cleanliness throughout the preliminary preparation of the formula, rapid cooling of formula as soon as heating is completed, and proper refrigeration. Any departure from the usually recommended procedure for formula preparation may be attended by the risk that the formula will become grossly contaminated.

2. There is no practical method of formula production which can assure sterility continuously in every bottle of formula. Certain organisms (particularly spore-forming bacteria) may survive regardless of the method used. The term "terminal sterilization" as applied to the usual home methods of formula preparation is a misnomer, since formulas may or may not be sterile after terminal heating.

3. The "aseptic" method of heating, although theoretically safe if carried out correctly, has the hazard that breaks in technique may occur so that pathogenic contamination may occur.

4. Unopened terminally-heated formula, when properly prepared and stored at a temperature of 40 F, remains bacteriologically safe for at least three days.

5. Keeping terminally-heated formula at room (68 to 75 F) or incubator (98.6 F) temperatures for more than six hours results in gross contamination of the formula by bacteria which had been dormant in the milk-mixture.

6. Although it is common practice in the home to prepare the required formula every day, our experiments would indicate that properly cleaned, prepared, cooled, and stored terminally-heated formula is bacteriologically safe for at least two or three days. A new supply of formula need not be made every day.

Butanol-Extractable Iodine Values in the Offspring of Diabetic Mothers. HENRY K. SILVER, Yale and Colorado.

Infants born of women with diabetes mellitus often present serious problems and have an increased morbidity and mortality. Studies of these infants and of the mothers during their pregnancies have failed to reveal the pathogenesis of the phenomena that occur among the infants. It has been suggested that such changes are related to a disturbance of maternal sex hormones, but the exact alterations or specific manner in which they affect the infant have not been determined. The present report is concerned with studies of butanol-extractable iodine (BEI) values in the serum of these infants and a discussion of the possible mechanism producing altered levels of the BEI.

It is known that the BEI rises in the normal child during the first few days of life with the limits being between 7μg. and 13μg. per 100 cc. on the fifth day of life. In a series of infants born to women with diabetes, abnormally low levels of BEI were found in six out of seven cases. In three of them the levels were under 4.5μg. per 100 cc.

No ready explanation is avilable to explain the low BEI at 5 days of age that was found in our patients. It may be that the pregnant woman with diabetes mellitus produces an excessive amount of various hormones of the anterior pituitary. Such overproduction may continue throughout pregnancy or cease gradually or abruptly at any time during gestation. When maternal stimulation ceases, there would be a temporary hypofunction of the infant's endocrine system. The "Cushingoid" picture which some of these infants exhibit at birth could be the result of excessive production of maternal hormones, while the lowered BEI at 5 days of age and the picture suggestive of adrenal crisis sometimes seen in such patients might be two of the manifestations resulting when secondary hypofunction of the infant's endocrine system occurs. The "physiologic hypothyroidism" which our patients demonstrated might be analogous to the physiologic hypoparathyroidism believed to occur in some newborn infants.

The Influence of Thermal Environment upon Survival of Premature Infants in the First Five Days of Life. WILLIAM A. SILVERMAN, JOHN W. FERTIG,* and AGNES P. BERGER,* Columbia.

A controlled clinical trial was designed to compare the effects of two levels of environmental temperature upon survival and respiratory signs in premature infants during the first five days of life. Among those who died during the trial period autopsy findings were compared. One hundred eighty-two infants admitted to the premature nursery of Babies Hospital from March, 1956, to February, 1957, whose birth weights were under 2000 gm., were enrolled in the trial before the comparison became definitive. Assignment to the study categories was made by random permutation within the weight groups.

The trial conditions were as follows: Incubator (air) temperature 83 to 85 F was designated as the "hypothermic" environment. Relative humidity between 80% and 90% was maintained in all incubators. Incubator temperature, axillary temperature, respiratory rate, and "retraction" score were recorded at six-hour intervals. Heart blood, lung puncture, tracheal swab, and cerebrospinal fluid cultures were obtained in infants who died during the trial period. Autopsy examination of infants who died during the trial period was performed without knowledge of the trial conditions which preceded the death.

A modification of the method of sequential analysis was utilized to evaluate the differences in survival under the two trial conditions. This sequential plan allowed a "running" analysis of weight-matched hypothermic-normothermic pairs. The plan was geared to be sensitive to an increase in survival rate in "normothermic" incubators ranging from 5% improvement to 10% improvement from levels of 90% to 75% survival, respectively, under "hypothermic" conditions. Previous experience of 15 months with "hypothermic" environmental conditions had indicated an expected survival rate ranging from 88% to 78%.

A significantly higher survival rate was observed in the "normothermic" group.

Characteristics of Human Proteinuria: The Significance of Variations in the Renal Clearance of Specific Plasma Proteins. ROBERT SLATER and D. W. GEIGER,* Toronto.

The alterations of kidney function productive of proteinuria in health and disease have been studied by comparing the renal clearances of specific plasma proteins. Sequential observations during persistent or intermittent proteinuria have been made in 11 children with the nephrotic syndrome, 6 patients with acute or chronic nephritis, and 7 children with orthostatic proteinuria.

Since previous electrophoretic studies of plasma and urinary proteins have revealed a considerable variation of protein components in different clinical conditions (some components have not yet been identified), the quantitative precipitin technique was used to define the transfer of albumin (M. W. 62,000), iron-binding globulin (M. W. 90,000), and γ-globulin (M. W. 160,000) from plasma into urine. Expressed as specific protein clearances, the data permit comparison of the renal handling of these proteins under the influence of such variables as plasma protein concentration, plasma osmotic and hydrostatic effect, glomerular membrane permeability, and tubular protein reabsorption. Furthermore, considered as protein clearance ratios (e. g., $C_{\gamma\text{-glob.}}/C_{alb.}$), the data may be expected to reflect any alterations in relative renal permeability to macromolecules independent of fluctuations in total protein clearance.

In patients with nephrosis, the clearance of albumin relative to the clearance of γ-globulin was observed to alter markedly during progression from relapse to remission or the reverse ($C_{\gamma\text{-glob.}}/C_{alb.}$ =0.01—1.18). In contrast, a "fixation" of this clearance ratio was noted during clinically static phases of active disease. Furthermore, in one patient with nephrosis the relative clearance of these proteins was not altered despite marked changes in the proteinuria induced by lordosis or albumin infusion.

Likewise, the findings in proteinuria of orthostasis in healthy children reflected a "fixed" state of relative renal permeability to macromolecules on repeated examinations in the same and different persons. The ratio $C_{\gamma\text{-glob.}}/C_{alb.}$ (0.5 to 0.8) remained steady, despite two-hundred-fold fluctuations in protein excretion during different periods.

In different types of proteinuria, the clearance of iron-binding globulin relative to the clearance of albumin remained constant ($C_{Fe\text{-glob.}}/C_{alb.}=1$) during simultaneous fluctuations in the relative clearance of γ-globulin. It appeared that the kidney showed no preference in the process of iron-binding globulin and albumin transfer into the urine.

Direct evidence is lacking in the human which permits differentiation between glomerular filtration and tubular reabsorption of protein. The finding of fixed relative permeability to macromolecules despite considerable fluctuation of renal protein excretion in different types of proteinuria is suggestive that any tubular reabsorptive mechanism is proportional and nonpreferential. If this assumption is true, then the nonparallel alterations observed in permeability to specific proteins ($C_{alb.}/C_{cr.}\%$; $C_{\gamma\text{-glob.}}/C_{cr.}\%$) during variations in the extent of renal damage in nephrosis may be

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ascribed largely to increased glomerular permeability to smaller-molecular-weight proteins (e.g., $C_{alb.}/C_{cr.}\%$ decreased 18 × while $C_{\gamma\text{-}glob.}/C_{cr.}\%$ decreased 3 ×). On the contrary, the proteinuria of nephritis and orthostasis apparently involves a more parallel increase in glomerular permeability to different-sized macromolecules.

Further Observations on the Amino Acid Requirements of Infants. SELMA E. SNYDERMAN and L. EMMETT HOLT JR., New York.

Observations have been continued on the requirements of infants for specific amino acids, using the technique described by Pratt et al. (*J. Nutrition* 56:231, 1955) in which a basal diet containing a mixture of 18 L-amino acids was altered by reducing the quantity of a single amino acid to find the minimal quantity that would permit weight gain and normal nitrogen retention.

Studies of the valine requirement showed that an intake of 85 to 105 mg. per kilogram of this amino acid was adequate.

Observations on arginine revealed no failure of weight gain or impairment of nitrogen retention when this amino acid was eliminated from the diet. Judged by these criteria, arginine thus does not appear to be essential for the growing infant. In the case of histidine, however, a definite requirement was found, between 30 and 35 mg. per kilogram being needed to sustain weight gain and normal nitrogen retention. This finding is in contrast to that in the human adult, for whom histidine does not appear to be an essential amino acid.

Genetic Combination of Types 1 and 2 Polioviruses. KATHERINE SPRUNT,* ISABEL M. MOUNTAIN,* WINIFRED M. REDMAN,* and HATTIE E. ALEXANDER, Columbia.

We reported previously at this meeting on the development of a combined poliovirus particle following the growth of Type 1 and Type 2 viruses within the same monkey kidney cell. The combined particles are of two types, one which contains predominantly Type 1 antigen with a smaller amount of Type 2 antigen and one which contains predominantly Type 2 with a smaller amount of Type 1 antigen.

Further work on these particles with use of the plaque technique shows that the combined virus is stable, as evidenced by study of the progeny of individual plaques, all of which retain the double antigenicity of the parent particles. It has been possible to carry out plaque-to-plaque passages without evident change in the resultant populations.

Progeny of doubly antigenic particles which have passed through five limiting dilution and four plaque-to-plaque passages can still be neutralized by both types of antisera and are capable of producing in monkeys neutralizing antibody to both types of virus in significant quantity.

It is suggested that the combined virus represents a stable genetic combination of the Type 1 and Type 2 antigenic factors.

Poliocidal Effects of Human Amniotic Fluid at Term. ALEX J. STEIGMAN and MURRAY M. LIPTON,* Louisville.

An interest in local immunity has led to a qualitative determination of γ-globulin in human amniotic fluid. Precipitin reactions using antihuman γ-globulin and control rabbit serums mixed with amniotic fluids indicated the presence of γ-globulin. Thereupon, anti-polio-virus titers of amniotic fluids collected at term simultaneously with cord blood and maternal blood serums were determined.

Amniotic fluids free of blood were obtained from women with unruptured membranes who delivered normally at term and from a cesarean section. Mothers' and their babies' cord bloods were also collected. Amniotic fluids and the blood serums were tested simultaneously for neutralizing capacity against the three types of poliovirus by the tissue-culture metabolic-inhibition color test. Undiluted amniotic fluids were tested for neutralizing capacity as well as twofold dilutions 1:2 to 1:512 against 100 TCD_{50} of each of the three types of poliovirus. These fluids were inactivated at 56 C for 15 minutes to 30 minutes. Twelve of twenty-two contained neutralizing substance against Type 1 virus ranging in titer from undiluted to 1:64; eighteen of twenty-one fluids neutralized Type 2 virus in dilutions ranging from undiluted to 1:256, and eleven of nineteen neutralized Type 3 virus in dilutions ranging from undiluted to 1:64.

In cases where amniotic fluid contained neutralizing activity, the corresponding maternal and cord sera had neutralizing titers ranging from 1:64 to 1:4096. In general, high amniotic fluid titers were associated with high maternal serum titers. The highest amniotic fluid titer observed was 1:256 against Type 2 poliovirus from a mother whose serum titer was 1:4096 against Type 2 virus. For each mother, the ratio of the serum-amniotic fluid titers appeared constant among the three serotypes of poliovirus.

In one concentration experiment 50% saturated ammonium sulfate was used to precipitate globulin from an amniotic fluid. The precipitate was redissolved in one-tenth of the original volume. Ideally, a maximum increase of tenfold neutralizing activity might be expected; approximately a fivefold increase was observed.

The protein content of amniotic fluid is reported to average 0.53 gm., with a range of 0 to 1.5 gm. per 100 ml., while the protein content of blood sera from the same patients varied from 4.07 to 7.5 gm. per 100 ml. (Cantarow, A.; Stuckert, H., and

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Davis, R. C.: *Surg. Gynec. & Obst.* 57:63, 1933). These comparative protein values would not entirely explain the discrepancy in antipolio titers between amniotic fluid and blood serums on a milligrams of protein basis. It is proposed to determine γ-globulin quantitatively in amniotic fluid in specimens titrated for poliocidal effects.

Studies of the Mechanism of Hematopoiesis: Relationship of the Mechanism to Leukemia. BERNHARD STEINBERG,* Toledo Hospital, Toledo, Ohio. Introduced by WILLIAM M. WALLACE.

That hematopoiesis is under the control of some mechanism has been suspected by many investigators in this field. The spleen, the adrenals, and the liver have been implicated in various degrees, but the problem remains resistant to effort, with very little light being shed on the nature of the mechanism which produces, distributes, and maintains the various blood cells. In our previous investigations it was indicated that aged leukocytes secrete into the circulation a substance, probably an enzyme (labelled expulsion factor), which acts on the marrow to expel leukocytes and replace those in the circulation and destroyed in the normal process of aging. (Steinberg and Martin, *Am. J. Physiol.* 161:14, 1950.) Subsequent studies suggested the presence of an "antiexpulsion factor," which held in check exuberant expulsion. Presence of these factors in the plasma of leukemic patients was investigated. Interpretations of the cellular changes were made possible by the study of the "Hematopoietic Response to Provocatives" (Steinberg and Martin, *A. M. A. Arch. Path.* 57:227, 1954). The present report is an extension of these experiments and is concerned chiefly with the action of certain fractions of human and bovine serum or plasma on the activity of the hematopoietic tissue of the bone marrow. Specific fractions selectively inhibit the production or maturation of granulocytes or of other marrow elements. Other factors induce an hyperplasia and an increase in maturation. On the basis of these findings a concept of a possible mechanism has been developed. It is postulated that each type of blood cell is controlled by a set of two hormone-like substances. One of them promotes cell development and maturation, and the other holds these properties in check. It is further postulated that damage to the latter substance allows an uncontrolled production of leukocytes and development of leukemia.

The Histopathologic Characteristics of the Liver in Congenital Atresia of the Bile Ducts. DANIEL STOWENS,* Walter Reed Hospital. Introduced by LEO GEPPERT.

Always included in the differential diagnosis of persistent jaundice in the neonatal period is congenital atresia of the bile ducts. Diagnosis of this not uncommon disease has in the past, and to a large extent in the present, depended upon visual inspection of the structures in the porta hepatis during laporotomy. This procedure, however, is neither certain nor always safe. Other means of diagnosis are therefore desirable.

Since the advent of needle biopsy of the liver diagnosis of this malformation by the alterations produced in the liver parenchyma is possible. This study, based upon the examination of material obtained from patients with congenital biliary atresia by biopsy and at autopsy, consists of a description of these changes and a classification of the disease into six varieties based upon the histopathological characteristics.

The histogenesis of the disease, as well as differential diagnosis, is also considered.

The Significance of Cytopathogenic Agents in Epidemic and Sporadic Diarrhea of Infancy. CYRIL S. STULBERG, WOLF W. ZUELZER, and ROBERT H. PAGE,* Michigan.

During the past few years research in diarrheal disease in infants has revealed the etiologic role of the enteropathogenic serotypes of E. coli. These organisms appear to be causative agents in the majority of the institutional epidemics that have been studied and account for approximately 25% of 459 sporadic cases of diarrhea admitted to the Children's Hospital of Michigan for treatment in 1955.

In our efforts to further delineate the etiologic significance of E. coli as well as the possible role of viral agents in epidemic and endemic diarrheal disease, the incidence of cytopathogenic enteric viruses was investigated in the infants involved in an epidemic due to E. coli O127:B8. The epidemiologic and bacterial studies of 60 cases occurring over a period of four months have been reported. From these, stool specimens were obtained from each infant during the acute phase of the disease and during convalescence. One hundred twenty-seven specimens were preserved at −20 C until each could be tested for cytopathogenic agents in a variety of tissue culture systems—monkey kidney cells and HeLa and Detroit-6 strains of human epithelial-like cells. From these specimens from infants ranging in age from a few days to 4 weeks, it is of significance that no cytopathogenic agents could be recovered. These results demonstrate that viral agents did not play an etiologic role in an epidemic caused by E. coli O127:B8. Forty-seven of the sporadic cases occurring in infants ranging in age from 2 weeks to 6 months have been studied thus far. From these, three cytopathogenic agents have been recovered.

Data on the isolation and identification of these agents and their relationship to enteric viruses and to diarrhea will be discussed.

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The Effect of Desoxycorticosterone Acetate (Doca) on Water and Electrolyte Distribution. A. Y. SWEET,* M. F. LEVITT,* and H. L. HODES, Mt. Sinai Hospital, New York.

Previous evidence has indicated that in dog and man the administration of adrenal hormones effects a temporary shift of sodium, chloride, and water in iso-osmotic proportions into the extracellular fluid. This conclusion was based on the assumption that inulin afforded a reliable measure of the volume of the fluid phase of the extracellular compartment. It has been contended that after the administration of adrenal hormones a molecule like inulin might enter areas from which it was previously excluded. In this event, the increased volume of distribution of inulin would not reflect an expanded extracellular volume but rather a change in tissue permeability to inulin. To test the validity of the original conclusion similar experiments were repeated in monkeys. In this protocol inulin spaces were measured before and after five days of administration of desoxycorticosterone acetate (Doca) to monkeys. As in the previous experiments cited, the animals were maintained on a low-salt intake so that any expansion of the inulin space could not be attributed to retention of salt. In these experiments, however, sodium, chloride, potassium, and water content of muscle were studied in control animals and in those subjected to Doca administration. Tissue electrolyte composition was measured to determine whether the expanded inulin space would be associated with a comparable increase in sodium and chloride content of muscle. Such a correspondence would strongly favor the hypothesis that the extracellular volume had actually increased. Alternatively, it would be difficult to accept the presence of an expanded extracellular volume without a proportional increase in the salt content of muscle.

It has been shown that following specific stimuli sodium and chloride of certain solid-phase extracellular tissues contribute to the electrolyte content of the extracellular fluid. Accordingly, the electrolyte and water composition of bone and tendon were measured in both control and hormone-treated monkeys.

Data will be presented which will show conclusively that a true increase in extracellular volume occurs following Doca administration. The effect of Doca upon the electrolyte composition of bone and tendon will be presented.

Muscle Nucleotides: Quantitative Isolation and Measure by Procedures Appropriate to Radiochemical Investigations on Small Tissue Samples. K. K. TSUBOI* and NATHAN EPSTEIN,* Cornell. Introduced by MAY G. WILSON.

A systematic development of appropriate analytical techniques was carried out prior to investigation of the role of nucleotides in the contractile processes of cardiac and striated muscle. Methods were developed for the quantitative isolation and measure of nucleotides by procedures yielding radiochemically pure products, using small excised muscles and muscle strips (of the order of 100 mg. of tissue). Heretofore existing methods (i. e., anion-exchange column chromatography) did not allow fractionation of these compounds in small quantities and in radiochemically pure form.

Methods have been developed for preliminary quantitative isolation of the nucleotides as a group, free for the most part from tissue contaminants and in high radiochemical purity, followed by microfractionation by two-dimensional paper chromatography (leading to apparent radiochemically pure products). Details concerning the isolation procedures, measuring devices, and procedures for quantitative absorption and radioactivity measurements of nucleotide deposits in paper will be described. In addition to the familiar adenine nucleotides, several unknown as well as many other known nucleotides have been detected as normal components of muscle cells. The techniques as developed for the isolation, fractionation, and measure of nucleotides can be applied effectively as well to tissues other than muscle and to various problems relating to these compounds.

Further Studies of Idiopathic Hypoglycemia. ROBERT A. ULSTROM and S. J. SANFILIPPO,* Minnesota.

Previous studies of children suffering from chronic idiopathic hypoglycemia by McQuarrie et al. have demonstrated the usefulness of adrenocorticotropic hormone in maintaining the blood sugar values at normal levels over long periods of time. The present investigation is an attempt to define more precisely the mechanisms responsible for the hypoglycemia and those which influence its amelioration by this therapy.

Ten children ranging in age from 2 months to 8 years have been studied by present methods. Seven of these children have been included in earlier reports. All have had several seizures associated with measured low levels of circulating glucose, but three of the ten children demonstrated no fasting blood sugar levels below 50 mg. per 100 cc. during the two- to three-week period of this study. Only one of the children was receiving corticotropin at the time of the studies, and no steroid values are included on this child. Six of the remaining patients were receiving small daily dosages of 9^{1}-cortisone at the time of admission to these studies. This therapy was discontinued for the study period.

A wide variation was found in the circulating 17-hydroxycorticosterone levels (method of Nelson and Samuels). The range of control 8 a. m. blood

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levels in nine patients was from 4.7μg. to 33μg. per 100 ml. ($n=20$), with a mean value of 16.3μg. and median of 18.4μg. per 100 ml. Although the lowest blood steroid values were found in those patients presenting the most severe problems and symptomatology from hypoglycemia, the converse was not true, and an inverse correlation of blood sugar and steroid concentrations was not a consistent finding. Regardless of control level of blood 17-hydroxycorticosteroids, a rise occurred in all patients two hours after intramuscular administration of corticotropin. A wide range in the degree of change in blood level was present. Increases of from 116% to 350% of initial blood levels were seen, resulting from a range of rise in absolute values of 5.0μg. to 28.1μg. per 100 cc. The absolute change in blood level showed no correlation with the control level. Dosage and route of administration of corticotropin undoubtedly accounts for some of the variability noted. Nevertheless, from these data as well as measurements of urinary steroid responses, it appears that the adrenal cortical response to exogenous stimulation is quantitatively equivalent to that of normal children.

Hydrocortisone alcohol was administered intravenously to eight of these children over a 30-minute period (1 mg. per kilogram of body weight). Levels of blood 17-hydroxycorticosteroids were measured at timed intervals hourly for four hours. The rate of disappearance of free 17-hydroxycorticosteroid from the blood is a reflection of the combined rates of metabolic changes and excretion of the injected steroid and thus is a general guide, only, to the rate of tissue metabolism of this compound. In five of these children free steroid disappeared from the blood in a logarithmic fashion when plotted against time. The "half-life" of the injected steroid in the circulation was found to range from 57 to 120 minutes in these children. A second such series of hydrocortisone-loading tests was made in the same patients with a preceding intramuscular injection of epinephrine (0.03 mg. per kilogram). The circulating free steroid half-life under these conditions ranged from 72 to 120 minutes. Although the disappearance rate was slower than the mean normal rate for age, we do not feel that a significant difference has been demonstrated.

Following a report by Cochrane et al. that the ingestion of L-leucine as the free amino acid (0.15 gm. per kilogram) or in casein (1.5 gm. per kilogram) may precipitate attacks of hypoglycemia, seven of these children have been tested with both substances and three, with casein alone. Normal children have shown a slight rise of blood sugar when tested during the two-hour period after oral administration of the amino acid or protein. Four of these hypoglycemic children have shown definite decreases in blood sugar levels following both L-leucine and casein ingestion. A fifth child, who was receiving corticotropin at the time of testing, was found to have a decrease of blood sugar from 40 to 30 mg. per 100 cc. in one hour after L-leucine ingestion, and from 46 to 32 mg. per 100 cc. after casein feeding. Since a diet high in protein has been commonly prescribed for hypoglycemic patients, a reevaluation of this practice seems necessary. Postprandial hypoglycemia may, at times, be explained in this way. Two of the children showing hypoglycemic responses to L-leucine are being given diets containing minimum nutritional requirements of this essential amino acid, and an attempt to evaluate the importance of this mechanism in their over-all carbohydrate metabolism is being made.

The Dynamics of Exchange Transfusion with Respect to the Removal of Bilirubin. VICTOR C. VAUGHAN III and FROUZANDEH B. BRELIAN,* Temple.

Serial determinations of levels of serum bilirubin before and after exchange transfusions and multiple exchange transfusions indicate that only one-third to one-fourth of the total body bilirubin is available for removal by this technique as it is usually carried out. Crude estimates indicate that the bilirubin space approximates 40% to 50% of body volume.

The effectiveness of exchange transfusion in removing bilirubin from the body of the infant with hyperbilirubinemia depends upon the interaction of a number of factors, including the amount of blood transfused, the size of the infant, the speed with which the transfusion is performed, and the rapidity with which equilibrium is reached in the distribution of bilirubin between the vascular and extravascular compartments. Mathematical expressions have been derived which lead to consistent descriptions of observed phenomena.

The implications of these observations for the optimal use of exchange transfusion will be discussed.

Influence of Prior Active Immunization on the Presence of Poliovirus in the Pharynx and Stools of Family Contacts of Polio Cases. PAUL F. WEHRLE,* RALPH REICHERT,* ORY CARBONARO,* and BERNARD PORTNOY,* State University of New York, Syracuse. Introduced by J. B. RICHMOND.

In an attempt to further evaluate the influence of active poliomyelitis immunization upon the epidemiology of inapparent poliovirus infection, quantitative determinations of virus excretion have been attempted. Laboratory data were obtained from 67 persons. Twelve represented clinical paralytic poliomyelitis cases, confirmed by isolation

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and typing of polioviruses in cultures of monkey kidney cells. The remaining 55 persons were family contacts, of whom 14 had been immunized prior to the onset of the index case.

Stool samples and throat secretions were collected for virus isolation from family contacts. The throat secretions were obtained by a uniform technique at 3-day intervals for 12 days after hospitalization of the index case.

Polioviruses were present in the feces of 8 of the 14 previously immunized children and in 16 of 23 unimmunized children. Polioviruses were isolated from 29 of 50 family contacts of all ages. The distribution of fecal virus titers was similar in the two groups of children.

There were 3 of 52 pharyngeal cultures from the previously immunized group which yielded poliovirus, while 15 of 88 cultures from unimmunized children were positive, many in high titer ($\chi^2 = 3.92$; $P < 0.05$). It would appear that in inapparent infections previous immunization may influence the presence of poliovirus in the pharynx without preventing or altering virus multiplication in the intestinal tract.

Other interesting features were a decline in the frequency of virus isolation with increasing age and the absence of an association between tonsillectomy and virus in the pharynx. Only one person, an adult, failed to have demonstrable homotypic neutralizing antibody in the second serum sample, thus demonstrating again the uniformity of transmission of these agents in family groups.

Experience with Combined Corticotropin-Mechlorethamine (ACTH-Nitrogen Mustard) Therapy of Lipoid Nephrosis. CLARK D. WEST, Cincinnati.

After favorable reports of the use of combined corticotropin and mechlorethamine therapy in the treatment of nephrosis from Viola Startzman and Robert Mercer, of the Cleveland Clinic Foundation, this form of treatment was instituted at the Cincinnati Children's Hospital in December, 1955, as a substitute for intermittent cortisone therapy. Treatment has been with courses of cortisone or, more often, corticotropin, for albuminuria only, adding on occasion mechlorethamine at the end of a corticotropin course. Evaluation of therapy has been by records of daily urine analyses for albumin kept by the parents and the hospital.

Initially mechlorethamine (0.1 mg. per kilogram per day intravenously) was given on the 6th, 7th, 8th, and 9th days of a 10-day course of corticotropin. In patients who had been subject to repeated exacerbations and who had been treated in the recent past with large amounts of corticotropin or cortisone, this regimen produced remissions which were 10 to 12 times as long as would be predicted from previous experience with these patients. On the other hand, in patients with new nephrosis, or with old mild nephrosis, who had recently had no corticotropin or cortisone, this regimen did not influence the course of the disease and albuminuria returned quickly. Because the duration of the remission appeared to be related to the extent of corticotropin or cortisone "priming" prior to the use of mechlorethamine, mechlorethamine therapy has subsequently been used only on patients who have had 15 to 23 days of corticotropin therapy during the preceding month. Of seven courses of mechlorethamine which have been preceded by 20 or more days of corticotropin therapy during the previous 30 days, all have resulted in long remissions; of four courses preceded by 15 to 17 days of corticotropin two have resulted in long remissions.

In addition to producing long remissions, combined therapy has two other advantages. 1. Institution of mechlorethamine injection after six or more days of corticotropin therapy regularly produces a remission, even in patients who have previously required long courses of cortisone or corticotropin for diuresis. Of 14 episodes of albuminuria in patients without discernible renal damage, remissions occurred in all after institution of mechlorethamine therapy. 2. Combined therapy appears to reduce the severity of the disease in that when albuminuria does subsequently appear it usually responds to shorter courses of corticotropin or cortisone than were required previously.

In summary, corticotropin-mechlorethamine therapy, properly used, appears to be a great aid in the handling of the patient with severe nephrosis who has frequent exacerbations and/or is relatively refractory to corticotropin and cortisone therapy. In such a patient, a diuresis almost always occurs when mechlorethamine is combined with corticotropin and if sufficient corticotropin has been given the probability is great that the remission will be relatively long. It is possible that combination of mechlorethamine with other dosage or schedules of corticotropin, or with cortisone, would be more effective in producing long remissions than the regimen described. No untoward effects of treatment have been observed to date.

Maple Sugar Urine Disease. ROLAND G. WESTALL,* JOSEPH DANCIS, and SHELDON MILLER,* New York.

A familial disease characterized by a maple sugar odor to the urine, central nervous system symptoms, and early death has been previously described by Menkes and others (*Pediatrics* 14:462, 1954). We have studied a patient who died at 20 months, the longest survival on record. Amino acid analysis of a urine and of a blood sample obtained during the last weeks of life revealed an abnormal pattern.

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The three branched chain amino acids—valine, leucine, and isoleucine—were greatly elevated, whereas threonine, serine, and alanine were exceptionally low. There was also an elevation of methionine and a decrease in the cystine in the plasma.

Aminonucleoside Nephrosis in Rats. S. G. F. WILSON,* JUDITH PFEFFER,* GERALD NASH,* and WALTER HEYMANN,* Western Reserve. Introduced by SAMUEL SPECTOR.

Frenk, Antonowicz, Craig, and Metcoff have described a renal disease in rats, induced by the daily subcutaneous injection of the aminonucleoside of puromycin, which simulates the nephrotic syndrome in children. We have confirmed this work. Observations have been made upon the latent period which characterizes the delayed onset of this disease. It can be shortened or lengthened by increasing or decreasing the dose, and the interpretation of this delay as a latent period is evident from the observation that after the intravenous administration of a single large dose, there is still a delay of five days before the onset of proteinuria.

If rats survive the initial disease, they all recover. Subsequent readministration of the aminonucleoside produces a chronic renal disease manifested by proteinuria, hypoproteinemia, and hyperlipemia, with progression to death in three to six months from chronic renal failure with hypertension and azotemia.

As in the nephrotoxic serum disease in rats, neither corticotropin nor cortisone was effective in preventing the aminonucleoside disease, and cortisone had apparently no beneficial effect upon the chronic disease.

The development of this nephrotic disease is restricted to rats. We have not succeeded in producing renal disease with aminonucleoside in mice, guinea pigs, rabbits, and dogs. The aminonucleoside is 9(3-amino-3-desoxy-D-ribosyl)6-dimethyl aminopurine. Neither the 6-dimethyl amino-purine, nor the 3-amino-D-ribose (Stern; Gidez) produces renal disease in the rat. It, therefore, seems possible that the failure to produce a nephrotic syndrome by aminonucleoside administration to mice, guinea pigs, rabbits, and dogs depends upon the ability of these animals to hydrolyze this compound, an ability which rats may not possess. Experiments to substantiate this hypothesis will be discussed.

The Effects of 2,4-Dinitrophenol on Respiration. ROBERT W. WINTERS,* T. F. WILLIAMS,* J. R. CLAPP,* and L. G. WELT,* North Carolina. Introduced by NELSON K. ORDWAY.

The substance 2,4-dinitrophenol (DNP) is known to produce an increase in metabolic rate and a concomitant increase in pulmonary ventilation. This hyperventilation has been assumed to arise from chemical stimuli secondary to the accelerated metabolic rate. Data which raise questions about this assumption have been collected in the course of other studies of the effect of DNP on renal tubular reabsorption of water. Dogs were given DNP, 5 to 10 mg. per kilogram intravenously. Marked hyperventilation began immediately and continued several hours. Arterial blood, sampled before and 5 to 140 minutes after injection of DNP, showed a rise in pH and fall in pCO_2 (calculated from pH and CO_2 content corrected to 38 C) in every instance, before any significant change in body temperature. Mean maximal changes in four unanesthetized dogs, expressed as before/after DPN, were pH 7.385/7.490, pCO_2 37.3/29.0 mm. Hg. Eight animals under anesthesia showed changes of similar magnitude.

DNP thus induces such marked hyperventilation that respiratory alkalosis results. It is suggested that this degree of hyperventilation cannot be simply secondary to increased production of CO_2 but probably represents as well a direct effect of DNP upon the respiratory center or its peripheral afferent connections.

Studies of the sensitivity to pCO_2 and hydrogen-ion concentration were carried out in five anesthetized dogs during inhalation of various concentrations of CO_2. In all instances the slope of the curve relating ventilation to either pCO_2 or H^+ concentration was increased after administration of 5 mg. per kilogram of DNP. Furthermore, this effect occurred without any significant increase in body temperature.

These studies demonstrate the following similarities between the effects of DNP and of salicylate upon respiratory metabolism: (1) an increase in CO_2 production; (2) an even greater increase in ventilation, so that the arterial pCO_2 falls, and (3) an increase in the sensitivity of the respiratory mechanisms to pCO_2 and H^+. The possible relationship of these findings to the known effects of these agents in cellular metabolism will be discussed.

Controlled Study of Three Methods of Prophylaxis Against Streptococcal Infection in a Population of Rheumatic Children: I. Streptococcal Infection and Recurrences of Acute Rheumatic Fever in the First Two Years of the Study. HARRISON F. WOOD,* GENE H. STOLLERMAN,* ALVAN R. FEINSTEIN,* ILSE HIRSCHFIELD,* JEROME H. RUSOFF,* ANGELO TARANTA,* and RAYMOND C. HAAS,* Irvington House, Irvington-on-Hudson, New York, and New York University. Introduced by L. E. HOLT JR.

Four hundred children with unequivocal histories of rheumatic fever are being followed in a carefully controlled five-year study to determine the incidence of streptococcal infections and rheumatic

* By Invitation

recurrences under three different prophylactic regimens. In addition, the study is observing the natural history of rheumatic fever and rheumatic heart disease in the proven absence of recurrent streptococcal infections.

The patients were randomly allocated into three statistically comparable groups, with the following treatment schedules: (1) sulfadiazine, 1.0 gm. per day by mouth in a single dose; (2) oral penicillin, 200,000 units per day in a single dose, one-half hour before breakfast; (3) benzathine penicillin, 1,200,000 units in 2 ml. by monthly intramuscular injection.

Every patient was seen in clinic monthly. When β-hemolytic streptococci were found in the routine monthly throat cultures, they were grouped with an antiserum to the Group A Streptococcus. Serum from each patient was routinely taken bimonthly for ASO determinations. Whenever an elevation was found in the routine titer, a serial determination of antistreptolysin O, of antistreptokinase, and of antihyaluronidase was carried out.

The present data report the relative effectiveness of the prophylactic regimens of the first two years of the study. There were 310 patients in the study at the end of the first year and 384 at the end of the second, and the two completed years encompassed 6812 patient-months. During this time a total of 95 streptococcal infections were found in 87 patients—40 infections in the first year and 55 in the second. The over-all infection rate for the two years was 1.4/100 patient-months. The streptococcal infections were numerically distributed among the three prophylaxis groups as follows: benzathine penicillin 15, oral penicillin 36, sulfadiazine 44.

In the first two years there were 13 recurrences of acute rheumatic fever, of which 2 were manifested as chorea only. The 13 recurrences represented 15% of the 87 children who had one or more streptococcal infections, and were noted in 9 patients receiving oral penicillin and in 4 receiving sulfadiazine. There were no recurrences in the patients receiving benzathine penicillin.

The present apparent superiority of benzathine penicillin may be an inherent property of the medication or may be due solely to the fact that it assures prophylaxis maintenance, since it is given by injection rather than orally. Further investigations will attempt to correlate the fidelity with which oral prophylaxis is taken and the occurrence of streptococcal infections. No significant incidence of drug sensitivity has been observed in the first two years of the study, but its possible future development must be considered before conclusions are drawn at this time.

Neonatal Meningitis. M. ZIAI* and R. J. HAGGERTY,* Harvard. Introduced by R. C. ELEY.

Bacterial meningitis during the first month of life is rare. When it occurs, however, early diagnosis is difficult and the prognosis is generally stated to be poor.

The literature consists only of small series of cases. In order to elucidate some clinical facts, useful in early diagnosis of this disease, we undertook to analyze the relatively large number of such case records available to us at the Children's Medical Center, Boston, and The Boston Lying-in-Hospital.

Fifty consecutive cases of purulent meningitis, exclusive of those with obvious central nervous system malformations, occurring during the first month of life were reviewed. These were seen over approximately a 20-year period.

The analysis confirmed the predominance of coliform organisms as the commonest etiologic agent. Boys were affected twice as commonly as girls; yet the case fatality rate was similar in both sexes.

The most relevant single factor was abnormal pregnancy or delivery, which occurred in two-thirds of the cases.

Clinical findings were varied and nonspecific, lethargy, poor feeding, respiratory symptoms, vomiting, and convulsions being the commonest points in the history. Aside from the general poor appearance of these patients, specific signs of meningeal irritation or tense, bulging fontanelles were each present in less than one-third of the cases. In several patients these nonspecific signs and symptoms did not suggest the diagnosis and several days elapsed before examination of cerebrospinal fluid was attempted.

The white cell count in the peripheral blood was variable and of little help diagnostically.

Mortality has remained high even with the use of antibiotics. Follow-up of the survivors indicates that an excellent recovery can occur, but this is dependent primarily on early diagnosis and institution of therapy.

Other factors elicited in this analysis relating to the pathogenesis, diagnosis, and therapy of neonatal meningitis will be discussed.

* By Invitation

Case Reports

Proteus Meningitis

Report of a Case in a Newborn Caused by Proteus Mirabilis

HYMAN C. TOLMAS, M.D., and JACQUELINE WINTER, M.D., New Orleans

Meningitis due to Proteus is a relatively rare entity, its incidence in the newborn period being extremely low. In a review of the world literature by McKee,[1] there had been only 18 reported cases up to 1944. An additional five cases were described between 1944 and 1952.[2-5] Since 1952 there has been no reported case of Proteus meningitis in the neonatal period.

Proteus mirabilis is thought to be a fairly common inhabitant of the gastrointestinal tract; yet in a review of the literature it was found that there has never been reported a case of meningitis due to this organism.

The following is believed to be the first case of this type of meningitis to be presented in the American literature.

Report of Case

The patient, a full-term infant was delivered on March 26, 1957. Labor and delivery were spontaneous and uneventful. Birth weight was 8 lb. 6 oz. (4020 gm.), and the baby was found to be in apparently good health. Routine newborn care (cord ligation, penicillin ophthalmic ointment, and vitamin K) was administered.

On the fourth day of life, the patient's temperature spiked to 103 F and he became irritable. Physical examination at this time was negative except for evidence of a moist umbilicus. The possibilities of septicemia and/or meningitis were considered. Blood for culture was drawn; the spinal fluid was cloudy, with a cell count of 8400 WBC per cubic millimeter, 89% of which were polymorphonuclear cells and 11%, lymphocytes. Smear of the spinal fluid demonstrated many gram-negative pleomorphic bacilli. Chemistry studies revealed a protein level of 210 mg., chloride level of 726 mg., and glucose of 41 mg. per 100 cc.

The blood picture disclosed a red blood cell count of 4,990,000 per cubic millimeter, hemoglobin of 15.8 gm. (109%), and white blood cell count of 6800 per cubic millimeter, with 30% lymphocytes, 46% segmented cells, 11% stab cells, and 13% monocytes. Bleeding time was 50 seconds, and clotting time was 4 minutes 41 seconds. The urine was amber, with a specific gravity of 1.010; sugar, 1+, and a trace of albumin. The microscopic examination showed many hyalin and finely granular casts.

An infusion of 5% dextrose in water for injection was started, and the patient was given 1,000,000 units of penicillin intravenously, dihydrostreptomycin 150 mg. intramuscularly, and chloramphenicol (Chloromycetin) 200 mg. intramuscularly as initial therapy. Five hundred thousand units of penicillin and 50 mg. per kilogram of body weight of chloramphenicol and of dihydrostreptomycin were given as maintenance therapy.

Blood and spinal fluid cultures yielded P. mirabilis. Sensitivity studies demonstrated that this organism was resistant to chlortetracycline (Aureomycin), erythromycin, chloramphenicol, penicillin, polymyxin B, oxytetracycline (Terramycin), carbomycin (Magnamycin), tetracycline, oleandomycin (Matramycin), methenamine mandelate (Mandelamine), sulfisoxazole (Gantrisin), and sulfisoxazole plus phenylazo-diaminopyridine hydrochloride (Azo Gantrisin), but it was sensitive to dihydrostreptomycin in concentrations of 5γ, 10γ, and 30γ; to neomycin, 30γ, and to novobiocin (Albamycin) in concentration of 100γ.

Penicillin was discontinued and novobiocin (100 mg. per kilogram) was started on March 30, 1957. The patient remained afebrile for four days following onset, having a temperature of 102 F on only one occasion. Except for having hyperirritability, when manipulated, the patient remained clinically asymptomatic, sucking well and retaining all feedings. He was given periodic doses of pheno-

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barbital (Luminal) sodium for irritability. On the third day after institution of therapy physical examination revealed a bilateral catarrhal otitis media, which had not been evident previously.

On April 4, 1957 (sixth day), a repeat blood culture was negative. Follow-up blood study disclosed a hemoglobin of 19.6 gm., hematocrit value of 62%, and a white blood cell count of 21,800 per cubic millimeter, with 58% lymphocytes, 13% stab cells, 18% segmented cells, 2% monocytes, and 9% eosinophils.

Chloramphenicol was discontinued on the seventh day. On April 8, 1957 (10th day) spinal tap showed clear fluid with a cell count of 19 white blood cells, all lymphocytes. Culture was negative. A left myringotomy was performed on this day, and a small amount of serous fluid was obtained. Culture of this exudate was negative.

Dihydrostreptomycin was discontinued on April 10, 1957, after the patient had been afebrile for eight days. Tympanotomy of the right ear was performed on April 11, 1957, with no exudate present.

The patient was discharged on April 12, 1957, apparently well and with no evidence of residual.

Comment

The rarity of this type of meningitis is well brought out by Carson and Koch's [6] report of only 1 case of Proteus meningitis in 354 cases of purulent variety over a period of 10 years. Approximately two-thirds of the cases reported in the literature were secondary to otitis media or mastoiditis.[7,8] It occasionally occurs as a complication of septicemia from a focus of infection, usually in the genitourinary tract.[9] However, in the case presented here, it is felt that the otitis evident on the third day was secondary to the septicemia. It is our belief that the organism causing the septicemia and meningitis may have been introduced by way of the umbilicus, but this was not cultured initially.

In an effort to determine the source of infection, blood, urine, and stool cultures were taken from the mother. P. mirabilis was found to be present in abundance in the stool. This case demonstrates well the importance of suspicion of meningitis and septicemia in the newborn infant in spite of the absence of neurological signs and symptoms of meningeal infection.

The use of streptomycin in the treatment of Proteus meningitis has materially reduced the mortality and morbidity rate. Before the advent of this antibiotic, therapy chiefly consisted of mastoidectomy, sulfonamides, bacteriophages, and vaccines.[1,8,10] Darnley [5] reported a cure of a case of Proteus meningitis with chloramphenicol and streptomycin. Because of the improbability of attaining a therapeutic blood level of 100γ of novobiocin, we are of the opinion that dihydrostreptomycin was chiefly responsible for curing this patient.

Summary

A case of septicemia and meningitis in a newborn infant caused by Proteus mirabilis is presented.

This is the only case of meningitis due to this specific organism reported in the literature.

A review of the literature is given.

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News and Comment

SOCIETY NEWS

The Society for Pediatric Research.—The meeting of the Society for Pediatric Research was held on June 19-21, 1957, at Carmel, Calif. The officers for 1957-1958, elected at the June 19 meeting, are as follows: President, Dr. Edward L. Pratt, Dallas, Texas; Vice-President, Dr. Alexander Nadas, Boston; Secretary-Treasurer, Dr. Sydney S. Gellis, Boston.

Members of the Council are Dr. Robert A. Aldrich, Seattle; Dr. Robert Cooke, Baltimore; Dr. Wallace McCrory, Philadelphia; Dr. John Osborn, San Francisco; Dr. Robert Slater, Toronto; Dr. Clark West, Cincinnati.

New members elected at the June meeting of the Society are as follows: Paul Adams Jr., St. Paul; Ray C. Anderson, Minneapolis; Henry W. Baird, Haverford, Pa.; William A. Blanc, New York; Charles W. Daeschner Jr., Houston, Texas; Robert W. Deisher, Seattle; Alan K. Done, Salt Lake City; Mary A. E. Engle, New York; Donald Fraser, Toronto; Eli Gold, Cleveland; Gustavo Gordillo, México, D. F.; Burton J. Grossman, Chicago; Charles Kennedy, Philadelphia; Barbara M. Korsch, New York; Loren G. MacKinney, Pittsburgh; J. Gordon Millichap, New York; M. Lois Murphy, New York; Arthur Parmelee, Pacific Palisades, Calif.; Robert Parrott, Washington, D. C.; William E. Segar, Indianapolis; James B. Sidbury Jr., Baltimore; Alan C. Siegel, Winnetka, Ill.; Thomas Sisson, Rochester, N. Y.; Mildred Stahlman, Nashville, Tenn.; Avron Y. Sweet, New York; R. Janet Watson, Brooklyn; Paul F. Wehrle, Syracuse, N. Y.; William B. Weil Jr., Cleveland Heights, Ohio; Robert W. Winters, Chapel Hill, N. C.; James A. Wolff, New York; Stanley Wright, Los Angeles.

The 1958 meeting will be held on May 6 and 7 at Atlantic City, N. J.

PERSONAL

Dr. Katherine Dodd.—Dr. Katherine Dodd has been appointed Distinguished Professor of Pediatrics, University of Louisville School of Medicine, effective August, 1957, it was announced by Alex J. Steigman, Chairman of the Department. Dr. Dodd is retiring as Chairman of the Department at the University of Arkansas, which she has served for the past five years. Previous to this, Dr. Dodd taught at the University of Cincinnati for nine years after teaching pediatrics at Vanderbilt for eighteen years.

As Distinguished Professor, Dr. Dodd will be free of administrative detail and able to bring to bear her rich experience in the teaching of clinical pediatrics upon the undergraduate students and the twenty-one house officers of the department.

Abstracts from Current Literature

American Journal of Medicine, New York

22, No. 3 (March) 1957

Chlorpromazine Jaundice: Analysis of 22 Cases. J. Lawrence Werther and Burton I. Korelitz.—p. 351.

Blood Carotene in Steatorrhea and the Malabsorptive Syndromes. Julius Wenger, Joseph B. Kirsner, and Walter L. Palmer.—p. 373.

* Hazard of Severe Infections in Splenectomized Infants and Children. Carl H. Smith, Marion Erlandson, Irving Schulman, and Gertrude Stern.—p. 390.

The Effect of Massive Prednisone and Prednisolone Therapy on Acute Leukemia and Malignant Lymphomas. Helen M. Ranney and Alfred Gellhorn.—p. 405.

Current Views on Pathogenesis and Therapy of Rheumatic Fever. Combined Staff Clinics.—p. 422.

Fifteen Years' Experience with Staphylococcus Septicemia in a Large City Hospital: Analysis of 55 Cases in the Cincinnati General Hospital 1940 to 1954. Rodman Wilson and Morton Hamburger.—p. 437.

* Scarlet Fever: Results of a Controlled Study of 609 Patients Treated with Penicillin and Sulfisoxazole. Alfred C. LaBoccetta, Morton Rosenberg, Susanna Harris, and T. N. Harris.—p. 458.

22, No. 4 (April) 1957

Electrolyte Studies in the Respiratory Paralysis of Poliomyelitis. A. E. Thompson.—p. 549.

The Relationship Between Pulmonary Infarction, Cor Pulmonale and the Sickle States. Kenneth M. Moser and James G. Shea.—p. 561.

Diagnosis of Ostium Primum Defects of the Atrial Septum. A. Calhoun Witham and Robert G. Ellison.—p. 593.

Deficient Thromboplastin Formation in Man. Marc Verstraete and J. Vandenbroucke.—p. 624.

* The Course and Prognosis of Reticuloendotheliosis (Eosinophilic Granuloma, Schüller-Christian Disease and Letterer-Siwe Disease): A Study of 40 Cases. Mary Ellen Avery, John G. McAfee, and Harriet G. Guild.—p. 636.

Hazard of Severe Infections in Splenectomized Infants and Children.—Carl Smith and his associates report on 19 children who developed serious infections 1 day to 12 years after splenectomy. These infections included seven cases of meningitis, eight attacks of acute pericarditis in five children who had had Cooley's anemia, one instance of acute endocarditis, three cases of bacterial sepsis, and three cases of acute fulminating infection in which no bacterial agent was recovered. The bacterial infections were not limited to any single organism, although of the 11 instances in which bacteria were recovered 6 were due to Pneumococcus. In general these infections occurred within two years of the splenectomy and developed in children who had not shown any unusual susceptibility to infection prior to splenectomy. No relationship was noted between the indication for splenectomy and the susceptibility to infection, the splenectomies having been performed for traumatic rupture as well as for accepted hemotologic indications. Serum γ-globulin determinations were made on eight patients to exclude hypogammaglobulinemia and showed, instead, greater than normal values, all eight levels being over 2.0 gm. That age may play a role in this increased susceptibility to serious infection is shown by the fact that only 1 instance of such infection occurred following 225 splenectomies performed on adults, whereas 14 serious infections developed among 50 children aged 13 months to 17 years who underwent splenectomy in the same hospital during the same 10-year period. Experimental evidence to demonstrate the possible roles of the spleen in body resistance to infection is cited. As further evidence of the incidence of serious infection in children following splenectomy the authors cite 14 similar cases found in the literature. Since these infections occur most commonly during the first two years post splenectomy, particular awareness of this risk should be made during this period.

Scarlet Fever: Results of a Controlled Study of 609 Patients Treated with Penicillin and Sulfisoxazole.—LaBoccetta et al. report on the comparative results of no therapy, sulfisoxazole, and penicillin in the management of 609 patients with scarlet fever, only 5 of whom were severely ill. Penicillin therapy over a five-day period resulted in a 50% reduction in the duration of fever as compared with the controls, in 99% conversion of

positive throat cultures to negative (only 30% conversion among controls), and in a 50% reduction in the incidence of bacterial complications (20.3% as compared with 45% among controls). In contrast, there was no significant difference in the results among the controls and among those receiving sulfisoxazole. Bacteriologic relapse or reinfection occurred in 8% of the penicillin-treated, in 16% of the sulfisoxazole-treated, and in 6% of the control patients. Significant rises in antistreptolysin titers between initial and three-week specimens occurred in only 20.5% of the penicillin-treated but in 47% of the controls and in 43% of the sulfisoxazole-treated. In the majority there was no further antibody rise between the three- and the six-week samples. Similar results were observed for antihyaluronidase titers, although serologic response to this test was less marked than with the antistreptolysin test. These studies are interpreted as reflecting the bacteriocidal action of penicillin in contrast to the bacteriostatic effect of sulfisoxazole.

The Course and Prognosis of Reticuloendotheliosis (Eosinophilic Granuloma, Schüller-Christian Disease and Letterer-Siwe Disease): A Study of 40 Cases.—Of 40 cases of reticuloendotheliosis, 2 manifested Letterer-Siwe's disease; 9, eosinophilic granuloma, and 29, Schüller-Christian disease. Although these three disorders may represent varying manifestations of an underlying pathologic process, the clinical features generally remain distinct, even though in some cases overlapping occurs. Letterer-Siwe's disease occurs in infants; has manifestations of visceral involvement of the lungs, liver, and pleen; has a hemorrhagic rash; frequently exists anemia, and usually is rapidly fatal. The nine cases of eosinophilic granuloma here reported represented largely asymptomatic solitary bone lesions in children over 4 years of age and in adults. Between these two symptom-complexes lies Schüller-Christian disease. Of note in the 29 cases of Schüller-Christian disease was the rarity (only 3 cases) of finding the complete classical triad of membranous bone lesion, exophthalmos, and diabetes insipidus. In 27 of the 29 patients skull lesions were demonstrable by x-ray, while in 16 the lesions could be palpated. Diabetes insipidus was present in 13, and although this symptom responded to vasopressin (Pitressin) it did not abate when the bone lesions healed. Exophthalmos, either unilateral or bilateral, was found 10 times. Of importance was the incidence (13 out of 29) of chronic otitis media, sometimes associated with radiologic changes in the mastoid or petrous pyramid. Skin lesions, usually eczematoid or seborrheic, were observed in nine patients and at times were the presenting complaint. Other common radiologic findings included involvement of the jaws (10 of 18 so x-rayed), lesions in the rest of the skeleton in 12 patients (especially pelvis, femur, and rib), and relatively asymptomatic pulmonary lesions. Of the 29 patients 4 have died; the 3 childhood cases demonstrated some visceral involvement and died within three years of onset. Eleven patients appear to have recovered, one having been well for 40 years now. The other 15 still show evidence of activity. Local x-ray therapy to osseous lesions, nodes, or subcutaneous masses was followed by improvement in 11 of 16. Likewise, steroid therapy was thought to have resulted in improvement in certain instances.

American Review of Tuberculosis and Pulmonary Diseases, New York

75, No. 4 (April) 1957

* The Effect of Isoniazid Therapy on Pyridoxine Metabolism in Children. Soledad M. Morales and Edith M. Lincoln.—p. 594.

Study of Tuberculin Protein and Polysaccharide Antigens by Gel-Diffusion Technique. Florence B. Seibert and Eva Soto-Figueroa.—p. 601.

The Properties of Para-Aminosalicylic Acid-Resistant Mycobacterium Tuberculosis var. Hominis. Michio Tsukamura.—p. 608.

75, No. 5 (May) 1957

* Significance of Bronchospirometric Values. G. Birath, S. Stallberg-Stenhagen, and E. W. Swenson.—p. 699.

Bronchospirometric Investigations Before and After Segmental Resection and Lobectomy for Pulmonary Tuberculosis. N. P. Bergh, G. Birath, and E. W. Swenson.—p. 710.

Bronchospirometry: VIII. Differential Function During Thoracic Surgery. T. R. Watson Jr., M. D. Tyson, M. L. Heller, J. J. Cincotti, and E. A. Gaensler.—p. 730.

Significance of the Terminal Rise in the Single-Breath Oxygen Test. J. K. Curtis and H. K. Rasmussen.—p. 745.

Quantitative Studies on the Multiplication of Tubercle Bacilli in Vivo. J. M. Robson and F. M. Sullivan.—p. 756.

The Significance of Bacterial Resistance to Drugs in Pulmonary Resections for Tuberculosis. David V. Pecora and Diran Yegian.—p. 781.
The Sensitivity of Human Leukocytes to Old Tuberculin. H. E. Hall and M. Scherago.—p. 807.
Disseminated Coccidioidomycosis. Jack Margolis.—p. 828.
Candida Albicans: A Means for Detecting M. Tuberculosis on Culture Media. E. Mankiewicz.—p. 836.

The Effect of Isoniazid Therapy on Pyridoxine Metabolism in Children.—It has been suggested that a pyridoxine deficiency, resulting from the antagonism between isoniazid and pyridoxine, may be responsible for neurologic manifestations observed in patients receiving isoniazid therapy.

The authors investigated the incidence of pyridoxine deficiency in a group of 26 tuberculous children. Twenty of these received isoniazid therapy for various periods of time. Clinical signs of deficiency, such as convulsions, anemia, or weight loss, were not observed, either in these children or in the other six tuberculous patients who served as one of the control groups.

The conversion of tryptophane to nicotinic acid has been studied in many animal species as well as in neurospora, and all evidence points to its dependence on kynureninase, a vitamin B_6-containing enzyme. In this study, the tryptophane-load test was used as a measure of vitamin B_6 deficiency, and the ability to convert tryptophane to N^1-methylnicotinamide was tested. Except in one case, all patients showed an increase in urinary N^1-methylnicotinamide excretion following tryptophane loading, thus confirming the absence of pyridoxine deficiency.

In contrast to the situation in adults, isoniazid in dosage used (5 to 20 mg. per kilogram per day) did not induce a pyridoxine deficiency in children. The authors conclude that prophylactic administration of pyridoxine during isoniazid therapy, as advised for adults, is unnecessary in children, but they suggest further studies on high dosage.

Significance of Bronchospirometric Values.—Factors influencing the values obtained at bronchospirometry have been reviewed and the following salient points are stressed:

If a gas mixture containing a high oxygen concentration is used in the spirometer system, a poorly ventilating lung may show a better value for oxygen consumption than it would if ambient air were used, but if concentrations used are about 50% to 60% the difference is negligible.

Even with the use of the largest size of the best catheter available at present (Carlens), turbulence prevails at even the slowest flow rates. The better a lung ventilates the more it will suffer by the resistance resulting from turbulence; this may give a contralateral poorly ventilating lung the appearance of being better than it really is. Obstruction due to secretions or anatomic changes of the left main bronchus may to some extent be corrected by judicious timing of the study, bronchial suction, and careful positioning.

As the blood flow through the pulmonary vascular bed varies according to posture, the oxygen uptake for a diseased lung can change in different body positions. By utilization of this phenomenon, information concerning the function within different parts of one lung can be obtained by investigations in the sitting and supine positions.

Double determinations of oxygen uptake and ventilation contributed by each lung agree only to a fair degree. Vital capacity with its attendant forceful respiration tends to overcome partial obstructions due to secretions and shows a smaller deviation.

In order to make a correct interpretation of the relative values obtained at bronchospirometry, these values must be properly related to each other and the bilateral composite. If the maximal function of the contralateral lung remains unchanged between two serial studies, and since the relationship between the two lungs tends to remain the same during work as well as at rest, the loss of function on one side can be calculated as percentage of its previous value. The function of the lung in question can best be followed if, after each bronchospirometric examination, its functional loss is calculated as a percentage of its normal value, for example, before and after a form of therapy has been instituted; this can only be done, however, if the opposite lung is not significantly involved in the disease process. Formulas for these calculations are given, and their derivations are presented.

American Journal of Mental Deficiency, Albany, N. Y.

61, No. 3 (Jan.) 1957

* Cultural Attitudes to Mental Subnormality. David H. H. Thomas.—p. 467.
The Role of Varied Therapies in the Rehabilitation of the Retarded Child. Dorothy Knight, Alice Jeanne Ludwig, Millicent Strazzulla, and Lillie Pope.—p. 508.

Infantile Spastic Hemiplegia, Intelligence, and Age of Walking and Talking, Meyer A. Perlstein and Philip N. Hood.—p. 534.

61, No. 4 (April) 1957

What Parents Expect the Institution to Do for Their Children. Margaretta Robbins.—p. 672.
What the Institution Expects of Parents. Malcolm Farrell.—p. 675.
Oligophrenia, Cerebellar Ataxia and Cataract. Ronald C. MacGillivray.—p. 719.
Comparative Study on Thorazine and Serpasil in the Mental Defective. George R. Sprogis et al.—p. 737.
* Reproduction in a Mongoloid: A Follow-Up. Grace M. Sawyer and Albert J. Shafter.—p. 793.

Cultural Attitudes to Mental Subnormality.—Present-day cultural attitudes toward subnormality are based on the "aggregation of impressions, concepts and affects resulting from the traditional cultural pattern" and the recent knowledge on the subject and its absorption into the fabric of the social pattern.

"The goal should never be lost sight of that eventually he [the subnormal] has a niche available to him . . . which it will be his privilege to fulfill in however lowly a plane."

With regard to present-day knowledge the author cites Fraser Roberts' work, in 1952, who established two types of subnormality: the higher-grade cases, derived from multifactorial additive genes of small unit significance, hereditarily determined, and the lower-grade cases, derived from single-gene pathological inheritance or major environmental incident. The first group, comprising most cases with an I. Q. of about 50, thus constitutes a normal biological variation. This fact is important because unless it is recognized the community will exclude an increasing number of persons with the lower levels of the normal variant as its pattern of civilization becomes more complicated.

In Britain about 10% of the school population falls into the category of educationally subnormal children (I. Q. of 50 or 55 to 80). A substantial proportion of this group can make good adjustment in the community after school. Further on we must consider the hypothesis of regression to normality of offspring of variants which, if accepted, would indicate that offspring of persons with intelligence levels below or above average will tend to show intelligence levels closer to the mean.

Reproduction in a Mongoloid: A Follow-Up.—A follow-up study of a now 18-year-old girl whose case was described in this *Journal* in October, 1949, seemed indicated because this physically, mentally, and emotionally healthy girl was the offspring of a then 25-year-old mongoloid mother. The child was the result of an incestuous relationship between the mongoloid girl and her father.

Two other cases of children born to mongoloid mothers are briefly cited, both resulting in mongoloid children.

The authors intend to follow the girl through adulthood to study her offspring and thus make further contributions to this puzzling problem.

Following this article Dr. C. E. Benda discusses the genetic, endocrinological, and external factors which may have etiological implications.

Canadian Medical Association Journal, Montreal

76, March 15, 1957

A Study of Infantile Colic. W. C. Taylor.—p. 458.
* Traumatic Hyphaema in Children: A Review of 113 Cases. M. Shea.—p. 466.
The Subnormal Child. P. G. Thomson.—p. 506.

76, April 1, 1957

Sexual Precocity: Report of an Ovarian Tumour in a Girl of 19 Months. C. J. Pattee, F. J. Tweedie, and A. K. Geddes.—p. 573.

76, May 1, 1957

* Celontin in the Treatment of Petit Mal in Children. J. S. Prichard, E. G. Murphy, and F. E. Escardo.—p. 770.
* Behaviour Problems in Juvenile Diabetics. P. Katz.—p. 738.

Traumatic Hyphaema in Children: A Review of 113 Cases.—During 1945 and 1955 there were 113 cases of traumatic hyphema seen at the Hospital for Sick Children, Toronto. Most cases (65) were caused by missiles (BB shot mainly); many (37), by sticks, belts, or

rope. Secondary hemorrhage occurred mainly on the third or fourth day after injury, and glaucoma most frequently occurred in cases with secondary hemorrhage. In the vast majority of cases the prognosis was good. Treatment is outlined.

Celontin in the Treatment of Petit Mal in Children.—The authors used a new anticonvulsant drug, Celontin, for 18 children with petit mal variant of centrencephalic seizures. The condition was completely controlled in three patients, and six achieved 75% control. Two patients were apparently made worse. The dose of the drug varied from 0.6 to 0.9 gm. in divided doses. Toxic effects, namely, rash and ataxia, are noted. Celontin appears to be another drug which is of value in certain cases of petit mal seizures.

Behaviour Problems in Juvenile Diabetics.—The author poses and answers four questions in this paper.

First, why be concerned with behavior problems or emotional disturbances in juvenile diabetics? It appears that behavior disturbances are relatively common in juvenile diabetics, and studies have shown that the diabetic status of a patient may vary with his emotional state because of alterations by the patient in his therapeutic regimen and because of metabolic reactions to situational life stresses.

Second, why is there an unusual incidence of behavior problems in juvenile diabetics? The answer to this question is perhaps relatively easy. The major etiologic factors in the incidence of behavior problems in juvenile diabetics seem to be, first, an unsatisfactory home situation made worse by the presence of a diabetic child and, second, the regimentation of the patient's life necessitated by the therapy of diabetes.

Third, were there many behavior problems in the staff juvenile diabetics at the author's hospital? Twenty-six such patients were observed, and in fifteen no evidence of behavior disturbance was present, but the remaining eleven, seven boys and four girls, did have behavior problems. Nine of the eleven behavior problem patients refused to follow their diets, and six manipulated their diabetes to achieve their own purposes.

Fourth, what can be done about behavior problems in juvenile diabetics? The author outlines the different points which will aid the physician and the patient, and the family. It is apparent that the physician must devote more time and effort toward establishing a good physician-patient-family relationship than in many other cases. The physician must play the role of the helpful understanding guide and not the punitive judge.

Blood, New York

12, No. 2 (Feb.) 1957

The Effect of Irradiation on the Plasma Erythropoietic Stimulating Factor. James W. Linman and Frank H. Bethell.—p. 123.
Recent Advances in the Knowledge of Total Red Cell Volume, Production and Destruction. Nathaniel I. Berlin, John H. Lawrence, and Paul J. Elmlinger.—p. 147.
Panels in Therapy: XII. Hypoplastic-Aplastic Anemia.—p. 193.

The Effect of Irradiation on the Plasma Erythropoietic Stimulating Factor.—Protein-free plasma extracts from rabbits made anemic by phenylhydrazine or repeated bleedings have been known to have erythropoietic stimulatory activity. When such extracts are administered to normal rats, red cell production is stimulated. The authors sought to determine whether x-irradiation would impair the production of this erythropoietic-stimulating factor and whether anemia due to irradiation alone would be a stimulus to its production.

Plasma extracts from rabbits made anemic by administration of phenylhydrazine beginning 24 hours after total body irradiation were found capable of stimulating erythropoiesis in the normal rat. Rabbits rendered anemic by total body irradiation alone were also able to produce the plasma erythropoietic-stimulating factor.

The authors conclude that the factor is not produced by hemopoietic or other radiosensitive tissue and its formation is not dependent upon a regenerative marrow.

Boletín médico de Hospital infantil, México, D. F.

13, No. 6 (Dec.) 1956

Mechanism of Therapeutic Action of Antibiotics in Brucellosis. M. Ruiz Castenada.—p. 975.
Evaluation of Medical and Surgical Services of Hospital Infantil of Mexico City. J. L. Perez Navarreta and Guillermo Sandoval.—p. 987.

Asthmatic Syndrome in Children. Luis Gomez Orozco.—p. 999.
Some Comments on Congenital Pes Equinovarus. Leanardo Zamudio.—p. 1023.
Poliomyelitis—A Systemic Disease. Fernando Lopez Clares, Enrique Rodriquez Diaz, and Carlos Mariscal.—p. 1039.

14, No. 1 (Feb.) 1957

Chemotherapy in Tuberculosis: A Study of Therapeutic Results of Use of Various Drugs in Children. Luis Gerlanza Berumen and Margarita Sales Quiroza.—p. 3.
Chemotherapy of Acute Leukemia in Children. Alejandro Aguirre, Mario Silva Sosa, Raquel Lopezlina, and Guillermina Berumen.—p. 19.
Neoplasms in Children. Maximilimo Salas M.—p. 39.
Post Traumatic Leptomeningeal Cyst. Miquel Ramos Murquina and Antonio Duque Medellin.—p. 63.
Clinical Case—Giant Cell Tumor (Original Technique of Arthrodesis). Francisco L. Mendoza Chavez and S. Romero S.—p. 73.
Clinical Pathological Sessions.

Chemotherapy of Acute Leukemia in Children.—The authors present the results of a study of 102 children, suffering from acute leukemia, who were treated with antimetabolites and corticosteroids. Aminopterin, 6-mercaptopurine, and cortisone were the specific drugs used.

Previous to treatment complete examination including adequate bone marrow punctures were made in order that there would be no doubt of the diagnosis and to get a homogenous group. Charts with details of the cases and results of the three types of medication and their use in combinations were made. When there was an infection cortisone was not used or, if it was used, proper antibiotics were given also and the K electrolytes were kept at proper level with KCl.

Data on remissions and length of survival are given. The frequency of remissions and therapeutic failures was the same in first, second, and third series of treatments (*80%* had remissions; *20%* were failures). Percentages of remissions were *63%* with folic acid antagonists, *88.6%* with 6-mercaptopurine, and *86.2%* with cortisone. Combinations of antimetabolites and cortisone did not affect the percentage of remissions but were valuable in certain specific cases.

There seems to be a group of cases amenable from the first to treatment, and those which resist therapy in first treatment continue to be resistant, although they may have as many remissions as those amenable to therapy.

Journal of Clinical Investigation, New York

35, No. 6 (June) 1956

On the Presence of Ferritin in the Peripheral Blood of Patients with Hepatocellular Disease. Kurt R. Reissmann and Mary R. Dietrich.—p. 588.
Diminished Phenylketonuria in Phenylpyruvic Oligophrenia after Administration of L-Glutamine, L-Glutamate or L-Asparagine. Alton Meister, Sidney Udenfriend, and Samuel P. Bessman.—p. 619.

35, No. 7 (July) 1956

The Interaction of Glucagon and Insulin on Blood Glucose. H. Elrick, C. J. Hlad Jr., Y. Arai, and A. Smith.—p. 757.
Thyroxine Metabolism in the Nephrotic Syndrome. Howard Rasmussen.—p. 792.

35, No. 8 (Aug.) 1956

The Effect of Thyroid Disease on Calcium Metabolism in Man. Stephen M. Krane, Gordon L. Brownell, John B. Stanbury, and Helen Corrigan.—p. 874.

35, No. 9 (Sept.) 1956

Studies on the Physiology of Hunger: I. The Effect of Intravenous Administration of Glucose on Gastric Hunger Contractions in Man. Albert J. Stunkard and Harold G. Wolff.—p. 954.
Hyposthenuria in Sickle Cell Anemia: A Reversible Renal Defect. H. G. Keitel, D. Thompson, and H. A. Itano.—p. 998.
Tracer Studies of the Absorption and Fate of Steroid Hormones in Man. Leon Hellman, H. Leon Bradlow, E. L. Frazell, and T. F. Gallagher.—p. 1033.

Journal of Pediatrics, St. Louis

50, No. 4 (May) 1957

A Comparative Evaluation of Sulfonamides. C. W. Daeschner, J. L. Clark, and E. M. Yow.—p. 531.
The Comparative Antipyretic Effect of N-Acetyl-P-Aminophenol and Acetylsalicylic Acid. M. T. Colgan and A. A. Mintz.—p. 552.

Anoxia as a Significant Perinatal Experience: A Critique. F. K. Graham, B. M. Caldwell, C. B. Ernhart, M. M. Pennoyer, and A. F. Hartmann.—p. 556.
Recurrent Staphylococcus Aureus Pneumonia and Empyema Treated with Novobiocin. H. Schneck.—p. 570.
* Serious Reactions to Novobiocin. R. A. Bridges, H. Berendes, and R. A. Good.—p. 579.
The "Growth Spectrum": A Simplified Chart for Assessment of Growth. G. M. Naimark.—p. 586.
Early Myositis Ossificans Progressiva Occurring in Homozygotic Twins. W. L. Eaton, W. S. Conkling, and C. W. Daeschner.—p. 591.
Recovery from Meconium Peritonitis Associated with a Diaphragm-like Obstruction of the Prepyloric Mucosa. A. J. Despirito and P. J. Guthorn.—p. 599.
Ventricular Septal Defect. H. Margulies, J. E. Gustafson, and J. T. McMillan.—p. 603.
Harlequin Color Change in a Young Infant with Tricuspid Atresia. H. A. Pearson and T. E. Cone Jr.—p. 609.
The Development of Sex-Role Inversion and Homosexuality. D. G. Brown.—p. 613.
Child Care in Iran. H. Ghadimi.—p. 620.
Acute Aseptic Myocarditis in the Newborn Infant. R. J. Blattner.—p. 644.

Serious Reactions to Novobiocin.—The authors review the literature on reactions to novobiocin. The literature gives an over-all reaction rate for adults of 12.7%, most of these having received 2 to 4 gm. per day for several days before the reaction occurred. The reactions have usually been allergic in type and associated with fever—urticaria, generalized erythema, or maculopapular eruptions; serum sickness has also been reported. Supposedly the therapeutic dosage of 15 to 60 mg. per kilogram per day is within safe limits. Little has been written concerning reactions in children. The authors report four cases, in one of which the patient died; also, they report on some experimental work.

The first case was a 5½-year-old child who was given 30 mg. per kilogram. On the eighth day a pruritic generalized maculopapular rash and elevation of temperature occurred. These disappeared when use of the drug was stopped. One month later one 250 mg. oral dose was given and several hours later a similar rash and fever and also edema of the face, hands, and feet appeared. An intracutaneous skin test with novobiocin was positive. The second case was a 15-year-old. After a week of novobiocin at 35 mg. per kilogram per day an erythematous blotchy rash occurred in association with fever. This stopped when the drug was stopped. The third case was a 6-year-old who was given novobiocin at a dosage of 40 mg. per kilogram. A week later a generalized pruritic maculopapular reaction arose, including dermal wheals. Liver-function tests and peripheral blood studies were all normal. The condition cleared up when the drug was stopped. The fourth case was a 14-year-old. He was given 2 gm. every 12 hours. On the eighth day jaundice developed. Elevated bilirubin and thymol turbidity were noted and also a prolonged prothrombin time. This boy died. Postmortem examination showed pulmonary edema, myocarditis, diffuse lymphoid hyperplasia, and a picture of acute diffuse necrosis of the liver. It was felt that this was very possibly a reaction to novobiocin.

Acute toxicity studies in dogs at a dosage level of 300 mg. per kilogram per day given intravenously have demonstrated severe prostration in two to four days. Necropsy revealed severe degenerative changes in liver and kidney. For 60 days at the same dose orally, two of three dogs developed some sulfobromophthalein retention. A dose of 200 mg. per kilogram per day given intravenously produced hemoglobinemia.

The authors conclude by saying that they feel that there is a high incidence of reaction to the drug and that one should keep this in mind when novobiocin is prescribed.

New England Journal of Medicine, Boston

256, No. 16 (April 18) 1957

Increased Erythropoiesis Induced by Androgenic-Hormone Therapy. B. J. Kennedy and A. S. Gilbertsen.—p. 719.
Medical Progress: Recent Developments in Glucagon Research. H. Elrick, A. Staub, and H. Maske.—p. 742.
Avulsion Fracture of the Tuberosity of the Ischium: Report of a Case. N. Gould.—p. 747.
Immediate Photographic Projection for Teaching. B. Appel.—p. 748.

256, No. 17 (April 25) 1957

* Therapeutic and Toxic Indexes of Digitalis: A Comparative Study of Gitalin and Digitalis Leaf. J. W. Bryfogle, T. Stantilli, H. A. Saltzman, and S. Bellet.—p. 767.
Effect of Sodium Phytate on the Hypercalciuria of Acute Quadriplegia Due to Poliomyelitis: Preliminary Report. P. R. Vagelos and P. H. Henneman.—p. 773.
Surgical Treatment of Lye Strictures of the Esophagus by Mediastinal Colon Transplant Without Resection. G. L. Nardi.—p. 777.
Medical Progress: Human Factors in Highway Safety; A Review and Evaluation. R. A. McFarland and R. C. Moore.—p. 792.

256, No. 18 (May 2) 1957

* Effect of Sulfonylurea Compounds in Diabetic Children. R. Camerini-Davalos, A. Marble, P. White, M. Belmonte, and L. Sargeant.—p. 817.
Prednisone Alone and in Combination with Salicylates and Phenylbutazone in the Treatment of Rheumatoid Arthritis. W. D. Platt Jr. and I. H. Steinberg.—p. 823.
Cardiovascular and Renal Complications of Cushing's Syndrome: A Clinical and Pathological Study of 17 Cases. D. A. Scholz, R. G. Sprague, and J. W. Kernohan.—p. 833.
Medical Progress: Human Factors in Highway Safety; A Review and Evaluation. R. A. McFarland and R. C. Moore.—p. 837.

256, No. 19 (May 9) 1957

Antibiotic Combinations: Antibacterial Action of Plasma of Normal Subjects after Oral Doses of Penicillin V, Tetracycline Hydrochloride and a Combination of Both. W. F. Jones Jr. and M. Finland.—p. 869.
Medical Progress: Human Factors in Highway Safety; A Review and Evaluation. R. A. McFarland and R. C. Moore.—p. 890.
Current Concepts in Therapy.—p. 900.

256, No. 20 (May 16) 1957

Relation Between Pyelonephritis and Bacterial Counts in the Urine: An Autopsy Study. R. A. MacDonald, H. Levitin, G. K. Mallory, and E. H. Kass.—p. 915.
* Platelet Transfusions in Pediatrics. J. J. McGovern.—p. 922.
Duplication of the Cystic and Common Hepatic Ducts, Lined with Gastric Mucosa: A Rare Congenital Anomaly. C. M. Lee Jr.—p. 927.
Medical Progress: Orthopedic Surgery. O. E. Aufranc, J. S. Barr, T. Brown, and E. E. Record.—p. 934.
Effect of Arginine and Glutamate on the Removal of Ammonia from the Blood in Normal and Cirrhotic Patients. S. P. Bessman, S. Shear, and J. Fitzgerald.—p. 941.
Prolonged Survival of Free Tumor Fragments. W. B. Patterson and H. R. Patterson.—p. 943.

256, No. 21 (May 23) 1957

Medical Progress: Orthopedic Surgery (to be concluded). O. E. Aufranc, J. S. Barr, T. Brown, and E. E. Record.—p. 991.
Reserpine and Extracellular-Fluid Volume. R. Melick and M. McGregor.—p. 1000.
* Atopic Dermatitis: A Discussion of Its Pathogenesis. J. S. Strauss and A. M. Kligman.—p. 1002.

256, No. 22 (May 30) 1957

A Study of Mitral Stenosis by Combined Catheterization of the Left and Right Sides of the Heart. J. Dickens, G. Raber, A. Woldow, G. C. Lorange, and H. Goldberg.—p. 1017.
Pulmonary Mucormycosis in Acute Histiocytic Leukemia. M. Stefanini and S. Allegra.—p. 1026.
* Blood Ammonia Levels in Hepatic Cirrhosis: Their Control by the Oral Administration of Neomycin. C. J. Fisher and W. W. Faloon.—p. 1030.
The Relation of Hydrocortisone Injections to Cleft Palate in Mice. T. H. Ingalls and F. J. Curley.—p. 1035.
Medical Progress: Orthopedic Surgery (concluded). O. E. Aufranc, J. S. Barr, T. Brown, and E. E. Record.—p. 1040.
Brief Recording: Anaphylactoid Reaction to Nitrofurantoin. A. A. Fisk.—p. 1054.

Therapeutic and Toxic Indexes of Digitalis: A Comparative Study of Gitalin and Digitalis Leaf.—The authors refer to the conflicting reports in the literature about gitalin in relation to other digitalis preparations. Some recent reports have claimed that the therapeutic dose of gitalin is only 40% of the toxic dose, whereas that of other preparations (digitoxin, digitalis leaf, and digoxin) is 60%. Others found no difference. The authors have attempted to establish this point by (1) animal experiments and (2) patient trial of gitalin compared with a glycoside whose use was well known.

In animal experiments the mean lethal dose in milligrams per kilogram in dogs of gitalin was 1.90 ± 0.09; for digitalis leaf it was 124.0 ± 3.0; for U. S. P. leaf it was 126.0 ± 7.0, and for digoxin it was 0.263 ± 0.016. In cats the lethal dose was lower for all preparations, demonstrating a species difference.

Clinical trials were conducted on patients with established atrial fibrillation whose ventricular rates and symptoms of heart failure had been controlled to a variable degree over a period of months or years. After a base line was established, one drug was used and then the other. Digitalis leaf was compared with gitalin. Usually doses were maintained for six weeks before they were changed, unless toxicity or the patient's condition warranted earlier change. All other cardiac medications were kept constant. There were 37 patients in whom the drugs could be compared. Full digitalization was considered to be shown by a persistent slowing of ventricular rate, to between 60 and 85 after 15 minutes' rest. This was further checked with atropine given intravenously, and if after atropine the ventricular beat was over 100 then full digitalization was not present. In 16 of the 37 cases full digitalization did not occur because

of toxicity. The dosages of each drug necessary to maintain comparable degrees of slowing were determined, and in 13 cases it was possible to compare toxic and therapeutic effects.

Results are as follows: 1. After 3.5 to 5 mg. doses of gitalin orally, it was found that onset of effect was from ½ to 2 hours, maximal effect was at 12 hours, and effect was waning by 24 hours. 2. For maintenance dosage it was found that about 0.5 mg. of gitalin was equivalent to 0.1 gm. of digitalis leaf but that maintenance in almost one-half the cases was higher with either than is usually considered necessary. 3. Both drugs had about the same effect in producing ventricular slowing. 4. Persons requiring larger doses of one preparation usually required larger doses of the other. 5. Toxicity appeared the same in each and seemed related to the potency of the preparation. Toxicity usually consisted of ventricular extrasystoles. The authors could not support previous observations of others that gitalin has a wider margin of safety.

Effect of Sulfonylurea Compounds in Diabetic Children.—The authors tested a group of children by the single-dose response method with Carbutamide and Tolbutamide, a method satisfactory in adults in determining probability of effect of the drugs on diabetics. Two hundred twenty-eight diabetics under 16 years of age were tested. They were on regular good diets. Two days before the test use of depot insulin was stopped and patients were maintained on crystalline insulin. On the morning of the test no insulin or breakfast was given. After blood was taken for determination of sugar, the drugs were given in a single oral dose of 1.5 gm. for ages 4 to 6 years, 2 gm. for ages 6 to 12 years, and 3 gm. for ages 12 to 15 years. Blood for determination of sugar was drawn at two and four hours afterward. A fall of blood sugar of greater than 20% when compared with the initial value was considered a positive result.

The results showed that the duration of diabetes and the insulin dose were the main factors that could be correlated with a positive test. Of 10 children with duration less than 6 months, 90% responded; of 11 children with duration between 6 and 12 months, 63% responded; of 18 children with duration between 1 and 1⅘ years, 44% responded; duration from 2 to 2⅘ years gave a 29% response. Of 28 patients with a dose of insulin of less than 20 units daily, 21, or 74%, responded (19 had had diabetes less than three years). Although there appeared to be a relation between fasting blood sugar level and response, in that if the level was 200 mg. or less there was more apt to be a response, the relation appeared really to be related to duration of diabetes. Also, although a blood level of 10 mg. or more seemed necessary for a positive result, there was no other correlation. Thirty-three children experienced untoward side-effects from a single dose of Carbutamide. There was nausea in 20, vomiting in 4, faintness in 3, dizziness in 2, abdominal pain in 2, weakness in 1, and convulsions in 1. With Tolbutamide there were side-effects in six cases—nausea in five (one with vomiting) and abdominal pain in one. There were no sequelae. Nineteen children were studied over a period of from one week to five months. One child developed a generalized pruritic eruption which disappeared after use of the drug was stopped. In no case did maintenance studies show a good result (70% of blood sugar values fasting, or more than three hours after eating, of below 110 mg. per 100 cc. was deemed necessary for good results). The authors feel that their studies suggest that these drugs do not work in the absence of insulin, since their good responses occurred in patients with early diabetes, who presumably has some working pancreatic tissue.

Platelet Transfusions in Pediatrics.—In this article the author discusses the use of platelet transfusions. The technique of collection was that of Gardner, Howell, and Hirsch, modified in that low temperature not used and ADC (acid-citrate-dextrose solution, National Intsitutes of Health Formula A) was used. The platelet concentrate was infused by means of plastic tubing and a specially prepared needle in a 10-minute period.

The bleeding time was corrected to normal or very nearly normal levels almost immediately. The platelet count rose appreciably by three hours. Improvement in the prothrombin consumption was correlated with the rise of platelets. It was found that it became almost normal when platelets rose to about 80,000 to 90,000 per cubic centimeter (phase method of counting). Duration of improvement in bleeding time was about one day, and rise in platelets lasted about three days. The mean platelet yield in terms of donors' platelets in the circulation was about 65%. In the short series no antiplatelet antibodies were noted after transfusion if they had not been present before. Active hemorrhage stopped within 15 to 30 minutes after transfusion in all of this small group. When overt bleeding again followed platelet transfusions, it was two or three days after laboratory values had returned to their abnormal pretransfusion

levels. When platelet transfusion was used before surgery no significant bleeding occurred during surgery or in convalescence. This method of treatment was used in the following cases: three cases of aplastic anemia and one of hypersplenism in preparation for surgery; five cases of leukemia with bleeding; three cases of idiopathic thrombocytopenic purpura with bleeding.

The author feels that one particular value of platelet transfusions is in preparing the patient with a low level of platelets for surgery.

Atopic Dermatitis: A Discussion of Its Pathogenesis.—The authors believe that the basic cause of atopic dermatitis is unknown. However, they do not believe that it is the result of atopy. In a set of experiments on allergic persons they demonstrated that painting the allergen (ragweed pollen) on normal skin produced no reaction nor was there a reaction of the skin when the allergen was later given intranasally or subcutaneously. If an area of skin was painted with Rhus toxicodendron, an area of dermatitis was produced. Then, if the allergens were given by local painting, intranasal distillation, or subcutaneous injection, six or eight persons developed an exacerbation of the dermatitis. Therefore, they concluded that an allergic mechanism may act in a secondary fashion on any primary dermatitis of unknown origin.

Blood Ammonia Levels in Hepatic Cirrhosis: Their Control by the Oral Administration of Neomycin.—When the relationship between blood ammonia levels and hepatic coma became known, attention was called to the sites of ammonia production. One large source appears to be the enzymatic action of bacteria on urea and other nitrogenous compounds in the gastrointestinal tract. In dogs neomycin was found to reduce the concentration of ammonia in blood draining from various intestinal sites. The authors report on the effect of the oral administration of neomycin upon the blood ammonia concentration and upon mental status in patients with cirrhosis.

Twelve studies were done on 11 patients with hepatic cirrhosis. These were divided into two groups. The first consisted of eight patients without neurologic manifestations on a diet of 110 to 120 gm. of protein. Blood ammonia levels were measured two hours after breakfast three times a week, the first being a control week. The second week the patients were given 3 gm. of neomycin every six hours (two patients received 2 gm. every six hours), and the third week was the post-neomycin-treatment week. Stool cultures were performed in six of the patients. The second group consisted of three patients with neurologic manifestations; they received 3 gm. of neomycin every six hours.

The eight patients in the first group all showed moderately elevated or high-normal ammonia levels. All demonstrated marked reduction of the ammonia level while taking neomycin. In the three patients of the second group there was likewise a marked reduction in the ammonia level regardless of the protein intake. The mental symptoms cleared. In one patient the ammonia level of the abdominal collateral venous drainage dropped sharply in 24 hours after start of neomycin therapy, whereas the ammonia concentration in the peripheral vein fell more slowly. In another patient, who had received neomycin for 12 days, there was no difference between the levels in the peripheral vein and in the portal vein obtained during a portacaval shunt procedure. Stool cultures in two patients revealed a normal intestinal flora after 12 hours of neomycin but only urease-negative yeast after 30 hours. The highest blood level of neomycin (in a patient with coexisting renal disease) was 0.12γ after three days of therapy (12 mg. per day).

The authors believe that neomycin reduces the blood level of ammonia in patients with cirrhosis by its effect on the bacterial flora. Mental improvement lags about 24 hours behind clinical improvement. The already existing level of blood ammonia is not affected, but must be removed by the liver or other tissues.

Pediatrics, Springfield, Ill.

19, No. 3 (March) 1957

Eczema Associated with Thrombocytopenic Purpura and Purulent Otitis Media: Report of 5 Fatal Cases. C. C. Huntley and S. C. Dees.—p. 351.

Severe Idiopathic Hypercalcemia of Infancy. G. L. Daeschner and C. W. Daeschner.—p. 362.

A Screening Test for Cystic Fibrosis of the Pancreas Using Analysis of Saliva. W. Hart and M. J. Naime.—p. 372.

A Case of Cryptococcus Neoformans Meningo-Encephalitis Complicating Boeck's Sarcoid. H. Lepow, L. Rubenstein, F. Chu, and J. Shandra.—p. 377.

Studies of Respiratory Insufficiency in Newborn Infants: II. Correlation of Hydrogen Ion Concentration, Carbon Dioxide Tension, Carbon Dioxide Content and Oxygen Saturation of Blood with Trend of Respiratory Rates. H. C. Miller, F. C. Behrle, N. W. Smull, and R. D. Blim.—p. 387.

Congenital, Multiple Peripheral Stenoses of the Pulmonary Artery. A. Gyllensward, H. Lodin, A. Lundberg, and T. Moller.—p. 399.

Comparative Frequency of Detection of Enteropathogenic E. Coli, Salmonella and Shigella in Rectal Swab Cultures from Infants and Young Children. M. L. Cooper, H. M. Keller, and E. W. Waters.—p. 411.

Studies on Ketosteroids in Urine of Normal Children Using Alumina Chromatographic Fractionation. M. Masuda and T. H. Holmes.—p. 424.

* Physiologic Studies on the Cardiovascular Status of Normal Newborn Infants (with Special Reference to the Ductus Arteriosus). F. H. Adams and J. Lind.—p. 431.

A Simple Technique for the Diagnosis of Nonlipid Histiocytosis. T. D. Moore.—p. 438.

The Incidence of the Nephrotic Syndrome in Children. M. B. Rothenberg and W. Heymann.—p. 446.

Trembling of the Chin—An Inheritable Dominant Character. B. J. Grossman.—p. 453.

The General State of American Pediatrics in 1855 with Particular Reference to Philadelphia. E. Caulfield.—p. 456.

Collaboration Between Pediatrician and Child Psychiatrist in a Rural Medical Center: Case Studies Illustrating the Role of This Relationship in the Postgraduate Education of the Family Physician. A. D. Hunt and M. Parmet.—p. 462.

Cytomegalic Inclusion Disease: An Analysis of the Clinical Features Based on the Literature and 6 Additional Cases. D. N. Medearis.—p. 467.

Recent Advances in the Management of Renal Disease in Children (Part I). W. W. McCrory and D. Macaulay.—p. 481.

19, No. 4 (April, Pt. 1) 1957

Pneumocystis Carinii—Etiologic Agent of Interstitial Plasma Cell Pneumonia of Premature and Young Infants. D. C. Gajdusek.—p. 543.

* Parenteral Administration of Reserpine in the Treatment of Hypertension Due to Acute and Chronic Nephritis: Clinical and Renal Hemodynamic Studies. C. W. Daeschner, J. H. Moyer, W. R. Bell, and J. L. Clark.—p. 566.

Tuberculous Meningitis Treated with Hydrocortisone and Cortisone. G. Szabo, V. V. Sterk, and S. Berman.—p. 580.

Galactose Tolerance in Glycogen Storage Disease. R. Schwartz, J. Ashmore, and A. E. Renold.—p. 585.

Infantile Hemangioendothelioma of the Liver: Report of 3 Cases. D. W. Crocker and R. S. Cleland.—p. 596.

Incidence of Abnormal Urinary Findings in Children Exposed to the Atomic Bomb in Hiroshima. M. P. Sullivan and Y. Takahashi.—p. 607.

Celontin (PM-396) in the Treatment of Epilepsy. S. Livingston and L. Pauli.—p. 614.

Repeated Psychometric Evaluations of Preschool Children with Cerebral Palsy. K. L. Kogan.—p. 619.

Ophthalmologic Findings in Children with Cerebral Palsy. W. S. Schachat, H. M. Wallace, M. Palmer, and B. Slater.—p. 623.

Recent Advances in the Management of Renal Disease in Children (Part II). W. W. McCrory and D. Macaulay.—p. 639.

Public Health, Nursing, Medical Social Work. M. E. Wegman.—p. 651.

Some Concepts of Plasma Protein Metabolism, A. D. 1956: E. Mead Johnson Award Address. D. Gitlin.—p. 657.

Early Recognition of Rheumatoid Disease with Comments on Treatment. R. D. Gauchat and C. D. May.—p. 672.

Prophylaxis of the Acute Asthmatic Attack in Infants and Children by the Use of Symptomatic Medication. J. Glaser and M. F. Smelzer.—p. 680.

A Regional Plan of Integrated Medical Services for Handicapped Children. E. B. Clark.—p. 685.

Bacterial Endocarditis. A. Dorfman.—p. 688.

Physiologic Studies on the Cardiovascular Status of Normal Newborn Infants (with Special Reference to the Ductus Arteriosus).—In an effort to delineate more accurately the physiologic status of the heart and great vessels in the neonatal period, eight newborn infants, believed to be normal, were studied by cardiac catheterization, electrocardiography, and determination of heart volume. The age of the infants at the time of study ranged from 7 hours to 14 days. It was felt that measurements of changes in heart volume were too few to establish conclusions regarding its variation with age, but values were noted to fall within normal values for age. Electrocardiograms were generally found to be within the range of normal for this age, with the exception of one infant displaying an unusually prominent R wave in Lead V_1. Data obtained from catheterization indicated the presence of pulmonary hypertension in the majority of infants studied, and it was felt that this pressure gradually diminished during the first week of life to values found in older children. A second important conclusion indicated by analysis of figures for oxygen saturation was that there is a significantly large left-to-right shunt of blood through the ductus arteriosus after the third day of life.

Parenteral Administration of Reserpine in the Treatment of Hypertension Due to Acute and Chronic Nephritis: Clinical and Renal Hemodynamic Studies.—In this study 44 patients with acute nephritis and 6 patients with chronic nephritis were evaluated as to

the response of their hypertension to the parenteral administration of reserpine; renal hemodynamic response to the drug was also studied in some of these patients. It is emphasized that reserpine is of value in acute hypertensive states only when given parenterally, since one to three weeks are required for maximum hypotensive effects when the drug is given orally.

Reserpine was given 64 times to the group with acute nephritis, and a fall in mean blood pressure was noted in all cases, the degree of effect apparently being directly proportional to the degree of hypertension. The effect was apparent within 1 to 3 hours and persisted generally from 12 to 18 hours. Significant decrease in blood pressure was similarly observed in patients with chronic nephritis. Renal hemodynamic studies showed a decrease in renal function associated with decrease in blood pressure, but this was not marked and was transient.

It was concluded that reserpine given parenterally in doses of 80γ to 150γ per kilogram of body weight is a safe and effective agent in treatment of hypertension associated with acute nephritis and that rarely the addition of hydralazine to the therapeutic regimen is necessary for ideal control of blood pressure. The fact that the reserpine effect appears to depend on the presence of hypertension and does not lower the blood pressure of normal subjects led to its designation as an antihypertensive agent rather than a hypotensive one.

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1. Breslow, Lawrence. "A Clinical Approach to Infantile Colic." J. Pediatrics **50** 196 (1957). A copy will be sent on your request.

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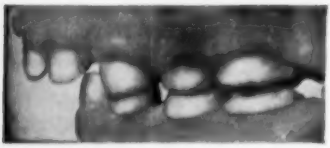

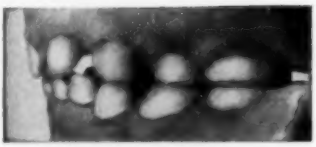

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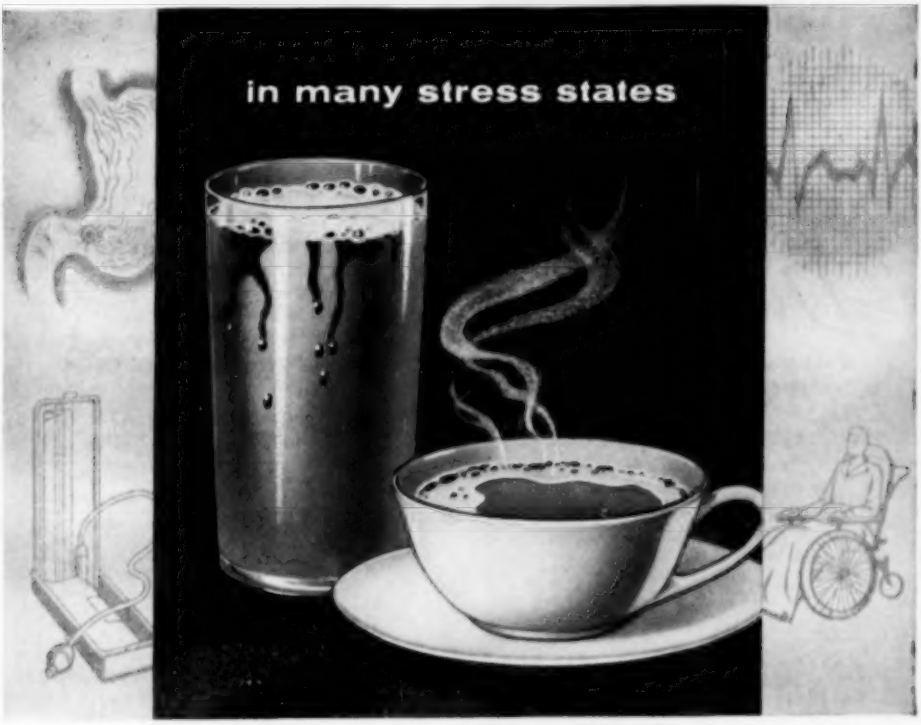

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Shwachman, H., The Child in Health and Disease, Grulee and Eley, 2nd Edit.

Shwachman, H. and Leubner, H., Advances in Pediatrics, VII, 249, 1955.

Kaaan, B. M., Illinois Medical Journal, 107, 120, 1955.

Kumar, B. B., and Gibbs, G. E. A.M.A. Journal Diseases of Children, 91:606, 1956.

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1. Odell, W. M.: Nutrition in Cardiovascular Disease, in Wohl, M. C., and Goodhart, R. S.: Modern Nutrition in Health and Disease, Philadelphia, Lea & Febiger, 1955, p. 699.
2. Bills, C. E.; McDonald, F. G.; Niedermeier, W., and Schwartz, M. C.: Sodium and Potassium in Foods and Waters, J. Am. Dietet. A. *25*:304 (Apr.) 1949.

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

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